BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

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VOLUME LXXXIX

BALTIMORE
THE JOHNS HOPKINS PRESS
1951

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VOLUME 89 NUMBER 1

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
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July, 1951

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PUBLISHED MONTHLY
THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland
Made in United States of America

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Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters and all remittances should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

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THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

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THE DIURETIC ACTIVITY OF COMPOUNDS RELATED TO XANTHINES, URACILS AND TRIAZINES AS DETERMINED IN DOGS.*

ALBERT A. KATTUS†, ELLIOT V. NEWMAN, AND JOHN FRANKLIN

(With the technical assistance of Marion Birmingham and Margot Robinson)

The Department of Medicine of The Johns Hopkins University School of Medicine and Hospital

Received for publication April 1, 1951

The need for a non-toxic diuretic drug which may be administered orally is apparent to anyone involved in the treatment of edematous patients. A possible approach to the problem of finding such a drug was suggested by the observation that the xanthine compound, theophylline, produced diuresis by specifically inhibiting the tubular reabsorption of sodium and chloride (1). Although the xanthines have been known for years to be non-toxic to the kidneys the low level of their diuretic potency has limited their clinical usefulness.

It was decided therefore to carry out a systematic search among chemical derivatives of the xanthines for compounds which might have more potent diuretic activity. A total of 41 compounds were therefore surveyed. Of these, 20 were related to the xanthines, 15 were uracil compounds, 3 were triazines, and 3 were complex ring structures. All were tested for diuretic activity in female dogs. Most of these compounds were synthesized by Papesch and Schroeder (2). In 1944 Lipschitz and Hadidian (3) reported the results of a similar survey in which they tested 70 amide and amine compounds for diuretic activity in the rat. From this survey formoguanamine, adenine, and melamine emerged as potent diuretics. These compounds are related to ours by virtue of the fact that they contain the N–C–N structure.

The following procedure was employed: The dogs were kept in metabolic cages and were fed a constant diet of Friskie dog meal. Water was allowed ad libitum. All urine was collected during 24 hour periods. Each collection period was completed by bladder catheterization. Two or three days control observations were made before the drug test day. The usual dose of the drug was 1 gram in 24 hours in the larger animals or .5 gram in 24 hours in the smaller animals. In a few instances tests were repeated with double doses of the drug. The test dose was always divided in two equal parts, put into capsules, one capsule being given by mouth in the morning and the remainder in the late

* Work supported in part by a grant from the G. D. Searle Company and from the Life Insurance Medical Research Fund.

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afternoon. Following the test day, collections were continued for one or two days in order to observe the recovery period. At least 4 days were allowed to elapse after the follow up period, before commencing the next series of control observations prior to testing a new drug.

Diuretic activity was assessed by observing the increase in urine volume on the test day over the average of the urine volumes during the two or three control days, and by observing the increase in sodium excreted on the test day over the average sodium excretion during the control days. Sodium was determined on an external standard American Cyanamid flame photometer having an accuracy of $\pm$ 2%.

The compounds tested and the results are shown in the accompanying table. Diuretic potency is expressed by averaging the urine volumes and the sodium excretions of the control days and determining what percent of these average values was excreted on the test day and on the first recovery day. Thus 200 would indicate that the urine volume or the sodium excretion on the test day had doubled over the average of the three control days, and 300 would indicate a tripling of the average control values. Percentages under 100, of course, would indicate urine volume or sodium excretion on the test day to be less than on the control days. The table shows all values expressed as percent of the control.

The figures for the first follow up day are given as a further check on diuretic activity because it is known that a pharmacologically induced sodium diuresis in a normal dog or normal human being is followed by a period of compensatory sodium retention during which time the presumably depleted extracellular fluid is replenished.

The consistency of the control data may be evaluated from the columns marked "control." Because dogs show wide fluctuations in urine volume and sodium excretion from time to time it was felt that one could not safely compare control data from various tests periods. It was, therefore, decided to treat each experiment separately. The variability between the data of the two or three control days is indicated by the percent variation between the urine volume and the sodium excretion of the last control day and the first when there were only two such days, and between the last and the average of the preceding two days when there were three control days.

This is expressed by the formula $\frac{\text{Na or UV last control day}}{\text{Average of preceding control days}} \times 100.$

In this way all values are expressed in percentages thus eliminating variations due to dogs of different size.

Since there was such marked variation between control days in many of the experiments it was felt that only very large changes on the test day over the controls could be considered significant. We have therefore arbitrarily decided that only compounds causing a sodium excretion of at least 150% of

TABLE I

COMPOUNDS	DOG	WEIGHT KG.	DOSE G/ DAY	**UV CONTROL %	*UV DRUG %	†UV FOLLOW %	††Na CONTROL %	‡Na DRUG %	§Na FOLLOW %	REMARKS
Xanthines										
Theophylline—(Dimethylxanthine)	Xm	18	.6	71	79	123	79	67	33	
	Sm	18	1.0	100	77	67	96	106	67	
	Sa	14	1.0	96	98	38	126	168	3	
Calcium Theophyllinate	Qu	16	1.8	81	212	99	92	450	18	
	Ha	7	.90	39	154	60	49	133	18	Vomiting
8-Chlorotheophylline	Sa	14	1.0	114	96	93	83	71	68	
3-Methylxanthine	Ha	7	.5	49	75	52	105	70	96	
	Ki	12	1.0	117	96		140	77		
7-Acetyltheophylline	Ti	7	.5	111	127	69	89	183	10	
	Ha	7	.5	150	100	76	107	90	37	
8-Acetylaminocaffeine	Je	12	.5	100	131	88	153	136	98	
	Di	6	.5	110	121	165	100	76	82	
8-Isoamyloxycaffeine	Sa	14	1.0	82	100	85				
	Sm	18	1.0	107	143	71	400	99	89	
Dimethyluric acid	Qu	16	1.0	94	88	84	103	79	94	
	Ti	7	.5	90	90	105	110	107	80	
1-Ethyl-3-methylxanthine	Ha	7	.5	97	250	80	84	185	33	Vomiting
	Ki	12	1.0	90	334	95	69	298	7	Vomiting
1-Ethyl-3-methyl-8-chloroxanthine	Qu	16	1.0	107	115	119	157	172	68	
	Ki	12	1.0	185	113	172	90	207	26	
1,3-Dipropylxanthine	Ha	7	.5		133	93		89	31	Vomiting
	Ki	12	1.0		190	62		140	13	Vomiting, 1 day control
1,3-Diethyl-2-thioxanthine	Qu	16	1.0		135	167		214	127	1 day control
	Ti	7	.5		131	42		140	22	1 day control
	Je	12	2.0	108	170	138	65	226	24	Vomiting
	Ha	7	1.0	82	170	82	122	200	15	Vomiting
1,3-Diethylxanthine	Sm	18	1.0	96	340	57	105	430	38	Vomiting
	Sm	18	1.0	135	400	115	119	540	20	Vomiting
	Mo	8	1.0	92	450	85	178	310	16	Vomiting
	Ki	12	.25	66	180	74	80	300	2	Vomiting
	Sm	18	1.0	93	240	160	79	500	11	Vom. } Given rectally
	Ha	7	.5	43	190	85	250	45	4	Vom. }
1,3-Diethyl-8-bromoxanthine	Sm	18	.5	100	135	111	133	190	74	Vomiting
	Ki	12	.5	193	230	140	68	203	38	Vomiting
	Qu	16	1.0	72	450	75	38	450	9	Vomiting
	Je	12	1.0	76	190	43	63	290	8	Vomiting
	Ch	6	1.0	118	190	111				Vomiting
	Sm	18	1.0	85	150	90	94	130	63	No vom. } Given rectally
	Ki	12	1.0	58	130	85	89	94	58	No vom. }
	Ha	7	1.0	85	96	77	95	96	38	No vom. }

** UV Control = percent variation between urine volumes on last control day and average of preceding days.

* UV Drug = urine volume on drug test day expressed as percent of average preceding control days.

† UV Follow = urine volume on day following drug test day expressed as percent of average control days.

†† Na Control = percent variation between 24 hour sodium excretion on last control day and average of preceding days.

‡ Na Drug = 24 hour sodium excretion on test day expressed as percent of average preceding control days.

§ Na Follow = 24 hour sodium excretion on day following drug test day expressed as percent of average control days.

TABLE I—*Continued*

COMPOUNDS	DOG	WEIGHT KG.	DOSE G/DAY	**UV CONTROL %	*UV DRUG %	†UV FOLLOW %	††Na CONTROL %	‡Na DRUG %	§Na FOLLOW %	REMARKS
Xanthines										
1,3-Diethyl-8-chloroxanthine	Qu	16	1.0	102	96	80	153	140	8	Vom. & Diarrhea
	Je	12	1.0	159	197	137	129	87	4	Vom. & Diarrhea
1-Methyl-3-ethylxanthine	Je	12	1.0	143	208	91	152	247	7	Vomiting
	Ha	7	1.0	133	218	109	90	152	22	Vomiting
	Je	12	.5	98	126	67	89	165	29	
1,3-Dibutylxanthine	Ti	7	.5	77	358	131	126	270	18	Anorexia
	Je	12	1.0	65	72	65	87	161	12	Vomiting
3-Butylxanthine	Qu	16	1.0	155	103	90	158	121	84	
	Ti	7	.5	212	195	89	154	264	60	
1-Methyl-3-butylxanthine	Qu	16	2.0	74	75	64	80	95	84	
	Ha	7	.5	95	57	76	83	33	85	
1-Ethyl-3-butylxanthine	Qu	16	1.0	166	170	110	103	206	18	Vomiting
	Je	12	1.0	68	95	78	38	140	7	Vomiting
Uracils										
1,3-Dimethyl-5,6-diaminouracil	Qu	16	1.0	55	144	114	97	114	149	Urine stained dark brown
	Ki	12	1.0	98	46	57	196	56	116	
1,3-Dimethyl-6-aminouracil	Sm	18	1.0	86	82	111	223	117	310	Dog ill—pyelonephritis
	Ha	7	1.0	40	74	53	228	145	55	
1,3-Diethyl-6-aminouracil	Qu	16	1.0	122	126	112	116	111	95	
	Ki	12	1.0	100	208	92	91	260	106	
1-Ethyl-3-methyl-6-aminouracil	Qu	16	.5	96	120	74	84	170	68	
	Ki	12	.5	133	185	70	95	158	30	
	Qu	16	1.0	77	150	87	73	166	80	
	Ti	7	1.0	70	112	83	63	125	62	
1-Isopropyl-3-ethyl-6-aminouracil	Qu	16	1.0	113	130	106	133	145	145	
	Ha	7	.5	84	154	103	65	140	140	
	Qu	16	2.0	90	82	70	80	65	15	Anorexia
	Ti	7	1.0	200	72	33	72	74	34	
1-Isopropyl-3-methyl-6-amino-2-thiouracil	Ha	7	.5	87	157	143	79	170	160	
	Je	12	1.0	86	124	77	80	180	85	
	Qu	16	2.0	118	103	99	94	118	107	
	Ti	7	1.0	119	117	65	44	118	93	
	Ha	7	1.0	140	172	120	160	162	108	
	Je	12	2.0	143	155	76	109	215	71	
1-(β-Hydroxyethyl)-6-aminouracil	Ti	7	.5	113	84	84	111	108	120	
	Ki	12	1.0	95	79	79	126	92	70	
1-(β-Hydroxyethyl)-5,6-diaminouracil	Je	12	1.0	176	116	110	275	230	260	
	Ki	12	1.0	116	134	108	108	97	103	
1-(β-Hydroxyethyl)-3-ethyl-6-aminouracil	Ti	7	.5		93	62		81	62	1 day control
	Ki	12	1.0		135	96		142	74	
1-(β-Hydroxyethyl)-3-methyl-6-aminouracil	Je	12	2.0	95	130	115	64	155	57	
	Po	12	2.0	91	50	145	64	139	108	
1-n-Propyl-3-ethyl-6-aminouracil	Qu	16	1.0	128	149	93	83	191	59	
	Qu	16	2.0	120	145	127	194	244	29	
	Ti	7	1.0	123	126	110	109	126	46	
	Di	6	1.0	143	176	68	122	218	23	
	Po	12	.5	194	182	120	129	142	40	

TABLE I—*Concluded*

COMPOUNDS	DOG	WEIGHT KG.	DOSE G/ DAY	**UV CONTROL %	*UV DRUG %	†UV FOLLOW %	††Na CONTROL %	‡Na DRUG %	§Na FOLLOW %	REMARKS
Uracils										
1,3-Dipropyl-6-aminouracil	Ha	7	.5	148	121	78	152	124	95	
	Je	12	1.0	122	145	69	183	182	24	
	Ha	7	1.0	94	70	58	187	55	34	Drug given on 2 consecutive days. Vom. on second day.
					99			157		
	Je	12	2.0	94	107	28	92	70	3	
					82			117		
1-n-Butyl-3-methyl-6-aminouracil	Ha	7	.5	122	151	98	130	20	8	Vomiting
	Ti	7	.5	71	190	58	100	178	2	Vomiting
1,3-Dibutyl-6-aminouracil	Po	12	.5	125	159	130	50	236	87	
	Ti	7	.5	58	94	83	23	48	47	
1,3-Dibenzyl-6-aminouracil	Ti	7	.5	87	110	116	74	141	134	
	Ki	12	1.0	130	106	102	102	131	126	
Triazines										
2,4,6-Trimethoxy-s-triazine	Sm	18	1.0	84	81	80	99	84	64	
	Sa	14	1.0	76	125	135	77	156	153	
2,4-Bis(propionylamino)-s-triazine	Sa	14	1.0	89	107	74				
2,4-Bis(acetamino)-s-triazine	Sm	18	1.0	64	192	133	25	153	47	
			1.0	99	156	107	85	133	99	
			1.4	119	268	157	123	159	94	
			1.5	121	152	92	45	233	33	
	Sa	14	1.0	92	535	256	114	225	66	
			1.0	98	380	154	108	168	44	
			1.0	102	400	180	92	246	9	
	Xm	21	1.5	111	315	347	105	94	34	
Ring Complexes										
10-[B-(B-Hydroxyethyl) methylaminopropionyl]-phenothiazine	Ha	7	.25	82	122	81	82	122	78	
	Je	12	.5	103	97	81	74	140	55	
N-(B-Dimethylaminopropionyl)-N-benzylaniline hydrochloride	Mo	8	.5	68	181	84				
10-(B-Dimethylaminopropionyl)phenothiazine hydrochloride	Sm	18	.5	64	65	81	35	240	27	
	Ki	12	.5	131	110	49	74	200	5	
	Ha	7	.5	87	250	93	91	163	9	

the control on the test day could be considered significantly diuretic. Sodium excretion has been considered a more reliable index of diuretic potency since it usually showed greater increase than did urine volume in compounds of high potency.

Compounds which we feel are definitely diuretic are shown in Table II in three groups based on sodium excretion potency. It should be emphasized

that the method used is a crude one suitable only for broad survey purposes to detect drugs of high diuretic potency. It is not presented as a means of titrating diuretic activity. Among the compounds not listed in Table II there are undoubtedly some which are appreciably diuretic but we do not feel the method to be sufficiently sensitive to be certain of these.

It will be seen from the data that several of the xanthine derivatives have extraordinarily potent diuretic activity. The most potent of all of these appears to be the 1,3-Diethylxanthine which increased the urine volume and the sodium excretion from three to five times over the control. However, this compound and its brominated derivative, also a potent diuretic, both caused repeated and severe vomiting which could not be prevented either by giving

TABLE II

Group 1—Sodium Excretion Potency over 400% of Control

1,3-Diethylxanthine

Group 2—Sodium Excretion Potency 200%—300% of Control

Calcium theophyllinate
1,3 Diethyl-8-Bromoxanthine
1 Ethyl-3-Methylxanthine
1,3-Dibutylxanthine

Group 3—Sodium Excretion Potency 150%—200% of Control

1,3-Diethyl-2-Thioxanthine
3-Butylxanthine
1-Ethyl-3 Methyl-8-Chloroxanthine
1-Methyl-3-Ethylxanthine
1-n-Propyl-3-Ethyl-6-Aminouracil
2,4-Bis(acetamino)-s-Triazine
10-(β-Dimethylaminopropionyl) Phenothiazine Hydrochloride
1,3-Diethyl-6-Aminouracil
1-Isopropyl-3-Methyl-6 Amino-2-Thiouracil
1-Ethyl-3 Methyl-6-Aminouracil

Compounds considered definitely diuretic arranged in order of decreasing potency as determined by increase of sodium excretion on test day over control.

the drugs rectally or by administering Dramamine prior to the drugs. Indeed, among all the xanthine compounds studied diuretic activity seemed to be parallelled by emetic activity.

Diethylxanthine was administered to three patients in doses of 100 mgms. three times a day and in each instance caused striking diuresis and severe vomiting. The diethylbromoxanthine was also given in similar doses to two patients, and in these there was no vomiting and no diuresis.

The uracil derivatives which were tested were in general much less potent in diuretic activity than the xanthine derivatives. However, among them there was much less evidence of gastro-intestinal disturbance. The compound

1-n-Propyl-3-ethyl-6 aminouracil appeared to have the greatest diuretic potency in this group.

Of the triazines tested one, 2,4-Bis(acetamino)-s-triazine, showed striking diuretic potency which appeared to be more specific for water than for sodium. This compound may also be called diacetyl formoguanamine and is closely related to formoguanamine which Lipschitz found to have great diuretic activity in the rat and dog (4), and which Ludwig (5) found to have diuretic activity in human patients. Because of the diacetyl derivative's marked potency in preliminary tests it was studied further. The results have been reported by Newman, Franklin and Genest (6). Potent diuretic activity was shown but profound diminution in renal function, crystalluria, and impaction of crystals in the renal tubules was demonstrated. The crystals were roughly spherical or ovoid in shape with concentric rings and spoke-like lines radiating from the center. They were similar in every way to those observed by Asper (7) following administration of formoguanamine to human patients, none of whom exhibited diuresis. One human volunteer who took 0.5 gram of diacetyl formoguanamine noted diuresis but there was sufficient crystalluria to discourage further use of the drug in human beings.

Compound 10-(B-Dimethylaminopropionyl) phenothiazine hydrochloride appeared to have significant diuretic potency. This was not tested further.

DISCUSSION

This survey indicates that minor modification of the xanthine molecule may produce compounds of great diuretic potency probably equal to the mercurials in this regard. The coincidental emetic activity of these compounds undoubtedly precludes their use as therapeutic agents. The low order of toxicity of the xanthines particularly toward the kidneys would suggest that such an agent might be highly desirable as a diuretic especially in the edematous patient with renal disease. The possibility that further modification of the molecule might preserve the diuretic activity and eliminate the emetic activity prompted a study of the uracil derivatives.

The uracils showed a very low order of emetic activity and one of them, 1-n-propyl-3-ethyl-6-aminouracil, showed good diuretic potency though not as great as diethylxanthine. This uracil was therefore selected for clinical trial. The results of this evaluation form the basis for another report (8).

The toxicity of the triazines as evidenced by the crystalluria and depression of renal function observed during administration of diacetyl formoguanamine precludes their use as therapeutic agents.

The mode of action of the uracils and xanthines is suggested by the fact that those with potent diuretic activity caused marked increases in the excretion of sodium. Potassium which was also measured showed no significant increase over the controls. These findings tend to indicate that the diuretic

activity of these compounds depends upon their ability to inhibit the reabsorption of sodium in the renal tubule. Support for this view may be found in the fact that in several of the tests the urine became hypertonic with respect to sodium on the day the drug was given.

It would be of great interest to know the exact chemical nature of the effect of these drugs on the renal tubular cells. Their biochemistry has not been investigated. Indeed in the absence of chemical methods for their determination we know nothing about their absorption, blood levels or excretion. It is conceivable that an investigation of these problems might lead to a better understanding of the physiology of the renal tubular cell.

SUMMARY

1. The diuretic activity of twenty xanthine compounds, fifteen uracil compounds, three triazines, and three compounds of unrelated structure has been studied in dogs.

2. Diuretic activity was determined by comparison of urine volume and sodium excretion on the test day with these values on control days.

3. Certain xanthine compounds showed high diuretic potency but this was complicated by emetic potency.

4. Diuretic potency was not as marked among uracil compounds but emetic activity was practically eliminated.

5. The most potent diuretic among the xanthines was 1,3-Diethylxanthine, among the uracils 1-n-Propyl-3-ethyl-6-aminouracil.

The helpful suggestions and criticisms of Drs. E. K. Marshall, Jr., and Margaret Merrell are greatly appreciated.

BIBLIOGRAPHY

1. Therapeutic Conference: The Treatment of Heart Failure, Part II. The Use of Diuretics. Bull. J.H.H. **81**: 430, 1947.
2. Papesch, V., and Schroeder, E. F.: Synthesis of Some 1-Mono-and 1-3 Dialkyl-6-Amino Uracils. Abstracts of the 117th Meeting of the American Chemical Society, April, 1950, Philadelphia, Pennsylvania.
3. Lipschitz, W. L., and Hadidian, Z.: Amides, Amines, and Related Compounds as Diuretics. J. Pharm. and Exp. Therap. **81**: 84, 1944.
4. Lipschitz, W. L. and Stokey, E.: The Mode of Action of Three New Diuretics, Melamine, Adenine, and Formoguanamine. J. Pharm. and Exp. Therap. **83**: 235, 1945.
5. Ludwig, H.: Uber die Diuretische Wirkung des 2, 4-Diaminotriazins am Menschen; vorlaufige Mitteilung. Schweiz. Med. Wchnschr. **76**: 822, 1946.
6. Newman, E. V., Franklin, J., and Genest, J.: Studies on the Diuretic Action of Diacetyl Formoguanamine in Dogs. Proceedings of the Meetings of the Johns Hopkins Medical Society. Bull. J. H. H., **82**: 409, 1948.
7. Asper, S. P., Jr.: Commenting on the above paper.
8. Kattus, A. A., Franklin, J., and Newman, E. V.: Clinical Observations on a New Oral Diuretic 1-n-Propyl-3-Ethyl-6-Aminouracil and Preliminary Studies on 1-Allyl-3-Ethyl-6-Aminouracil. To be published.

HISTOCHEMICAL STUDIES ON CARTILAGE AND BONE

II. Ascorbic Acid Deficiency[1, 2]

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Received for publication April 10, 1951

Ascorbic acid deficiency leads to profound changes in the skeletal tissues of susceptible species of which the guinea pig and man have been most extensively studied. It seems well established that one of the primary effects of a lack of this vitamin is to affect the metabolism of the osteoblast in some way so that this specialized cell is unable to promote the formation of bone matrix, i.e., osteoid. The ensuing morphological changes which are encountered and which provide us with the classical pathologic picture of skeletal scurvy result from mechanical forces and are a consequence of the fundamental disturbance in osteoblastic activity (4). The effects of ascorbic acid deficiency on epiphyseal cartilage are less clear; we feel that the changes found here result from a combination of nonspecific inanition and disturbances, mechanical in origin, resulting from absence of osteoblastic activity in the shaft beneath. This will be dealt with more fully below.

The present observations are a continuation of those which have been carried out on normal bone (5). Guinea pigs have been used as the experimental animal. They have been placed on scorbutogenic diets and either sacrificed when *in extremis* or treated for varying periods and then sacrificed, in order to study the reparative sequences. The histochemical methods utilized have been detailed elsewhere (5).

ENZYMES

Cytochrome oxidase: When the *nadi* reagent is applied to free hand slices of ribs from animals dying of absolute scorbutus there is either a reduction in or an absence of the formation of indophenol blue. Muscle tissue adherent to the bone gives a prompt and intense reaction while in the cells of the *gerüstmark* the pigment takes longer to appear and apparently these cells stain less deeply than normal osteoblasts which ordinarily give a prompt reaction, usually in advance of the muscle fibers. This delay and poorer quality in staining is also true of the periosteal cells as well. When healing is promoted

[1] Aided by a grant from the Florida Citrus Commission.

[2] A portion of the data presented herein was presented at the First and Second Conferences on Metabolic Interrelations of the Josiah Macy, Jr. Foundation, New York City, Feb. 7 and 8, 1949 (1) and Jan. 9 and 10, 1950 (2) and at the meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 17–21, 1950 (3).

by the administration of ascorbic acid, the osteoblasts after 24 to 48 hours exhibit normal reactions; that is there is a prompt appearance of blue staining granules in their cytoplasm. As we shall see below, such cells are promoting in some way the formation of fibers in their immediate vicinity. As is the case of normal costal or epiphyseal cartilage, these structures in scorbutic guinea pigs are not colored by the *nadi* reagent, indicating the absence of cytochrome oxidase activity.

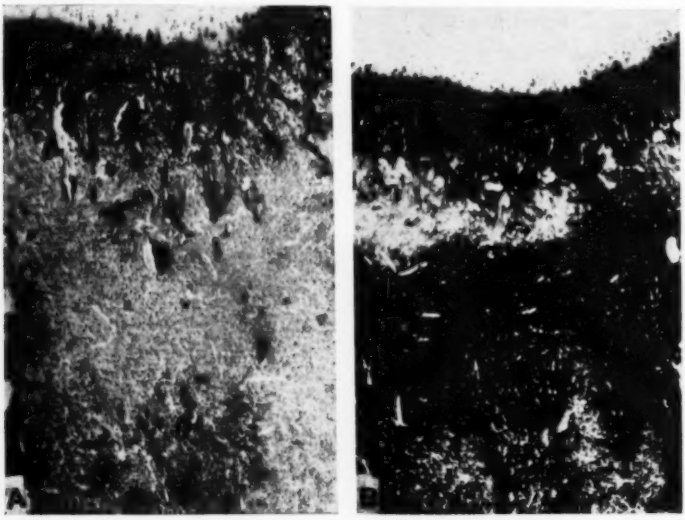

FIGURE 1. *A*. Low power photomicrograph of rib from guinea pig dying of absolute scorbutus. Alcohol-fixed, undecalcified section has been stained for alkaline phosphatase activity by the Gomori technique. Note presence of black staining material in the hypertrophic cells. Positive reaction is absent in the cells of the *gerüstmark*.

B. Low power photomicrograph (same magnification as *A*) of a rib of guinea pig allowed to develop scurvy almost to the point of death and then treated with ascorbic acid for 48 hours. Section treated as in *A*. Note presence of large amount of black staining material in the *gerüstmark*, indicating a return of phosphatase activity.

Phosphatase: In absolute scorbutus there is a complete absence of phosphatase activity in the *gerüstmark* (Figure 1). This area which is composed of spindle-shaped cells resembling fibroblasts, fragments of bone, bits of calcified cartilage matrix, and hemorrhage is sharply demarcated from the underlying marrow elements whose nuclei furnish a control in that these structures give a positive reaction. Twenty-four hours after the administration of 20 mg. ascorbic acid intraperitoneally a profound change is seen. The connective tissue-like cells comprising the *gerüstmark* have rounded up and their cytoplasm has become more prominent. Such cells exhibit an intensely positive reaction

FIGURE 2. High power of section shown in Figure 1, *B*. Note prominent staining of nuclei and the processes of the now physiologically normal osteoblasts and phosphatase positive intercellular fibers.

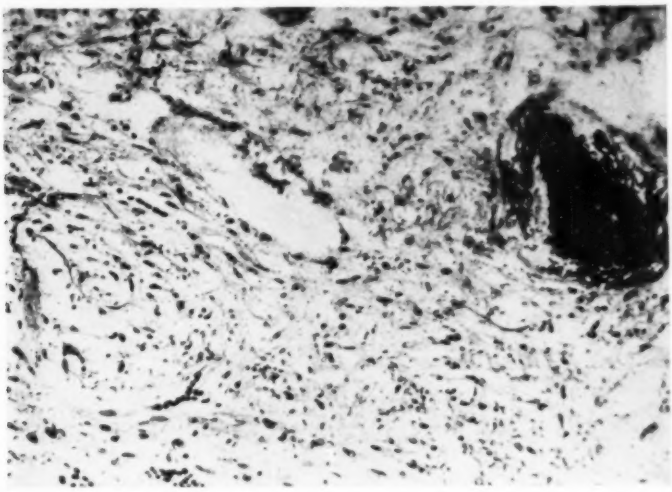

FIGURE 3. High power of *gerüstmark* in rib of an animal dying of absolute scurvy. Section has been stained with silver to bring out reticulum of which there is none. Only nuclei stain.

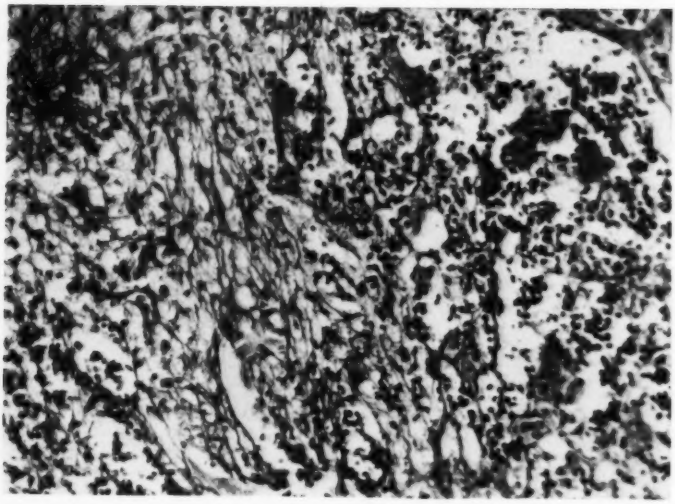

FIGURE 4. *Gerüstmark* of rib in an animal allowed to develop severe scurvy and then treated for 24 hours. Note appearance of argyrophilic fibers between nuclei.

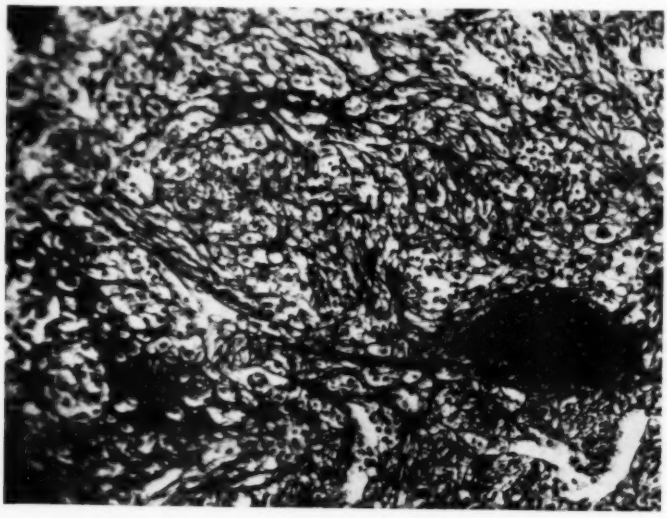

FIGURE 5. *Gerüstmark* region of an animal allowed to develop scurvy and then treated for 72 hours. Note extensive silver staining fibrils which in some areas have fused to form osteoid matrix. This does not stain black as do the fibers but has a reddish color.

so that one sees a mass of dark staining nuclei together with fine fibers nearby which also give a positive reaction (Figure 2). These intercellular fibers, as will be brought out below, are argyrophilic and metachromatic (Figures 3, 4

and 5). In absolute scorbutus the periosteal cells, which normally exhibit a positive reaction, also fail to show phosphatase activity. Upon treatment, as they begin to initiate the formation of bone matrix, positive staining, which denotes phosphatase activity, returns. In contrast, even in the most severe and absolute scorbutic animals the cartilage continues to display activity and this is in no way altered by therapy. One reservation must be made as far as cartilage is concerned. There may be an apparent decrease in phosphatase activity as a result of narrowing of the zone of hypertrophic cartilage cells due to mechanical factors. That is, because of separation of the cartilage from the shaft, the former stops growing. Inanition also tends to retard the poliferative activity of the cartilage cells.

CARBOHYDRATES

Glycogen: As is well known glycogen is a prominent constituent of the hypertrophic cartilage cells of the epiphysis or costal cartilage. This polysaccharide does not disappear from these cells in absolute scorbutus. A reservation must be made, however. As the animal ceases to eat and the effects of nonspecific inanition appear, there is a reduction in the amount of glycogen due to a reduction in the number of hypertrophic cells. So too, when there has been a mechanical separation of the cartilage on the shaft, growth of the cartilage may likewise be interfered with. This is the same phenomenon we referred to above.

The fibroblast-like cells of the *gerüstmark* do not contain glycogen. Upon adequate treatment, however, fine granules appear in the cytoplasm which can be shown to be glycogen by appropriate methods. The osteoblast normally, of course, contains this material as does also the osteocyte.

Mucopolysaccharides: Normally both cartilage matrix and osteoid exhibit metachromasia and stain positively with the periodic acid-leucofucsin technique. It is also plain that the fibrillar structure of these two matrices stains more deeply than the interfibrillary areas. In absolute scurvy there is no metachromatic material in the *gerüstmark*. The spindle-shaped cells themselves show minimal purple staining material in their cytoplasm. When scurvy heals fine fibers appear in the vicinity of the now functionally potent osteoblasts. The cytoplasm of the cells which have changed from spindle forms to plump almost epithelial-like structures is metachromatic. The fibers are metachromatic and also can be stained, though not deeply, by the periodic acid reaction. These fibers as will be brought out below are argyrophilic and can therefore be classified as reticulum. It will be recalled that the fibers also give a positive phosphatase reaction. Such fibers coalesce to form osteoid and in so doing the resulting osteoid loses its ability to stain with silver unless treated in special ways. As far as metachromasia and the periodic acid reactions are concerned, the rather prompt deposition of inorganic materials obscures these reactions in bone matrix.

Cartilage shows no reduction in metachromasia or in its reaction with periodic acid-leucofucsin even in the animal dying of absolute scorbutus. Due to mechanical defects in the underlying shaft, the epiphyseal plate or costal cartilage may become distorted and seemingly stretched, the lowermost border assuming a convexity so characteristic of the rib in human scurvy. The spreading apart of the cartilage cell columns reveals the fibrillary structure of the matrix in an admirable fashion.

NUCLEOPROTEIN

Ribose nucleic acid: As is well known the cytoplasm of the osteoblast is basophilic. This basophilia is apparently due to the presence of ribose nucleic acid (10). When skeletal tissues of normal animals are stained with methyl green-pyronin the cytoplasm of the osteoblasts lying along the trabeculae and of those just beneath the surface of the trabecula, i.e. the most recent osteocytes, stains a brilliant red. The cytoplasm of these cells is, of course, basophilic in ordinary hematoxlyn-eosin preparations. The spindle shaped cells of the *gerüstmark* in absolute scorbutus have virtually no red staining material in their cytoplasm. Although the amount of cytoplasm is reduced there would appear to be room for this material if it were present. One or more red staining nuculeolar globules in the elongated nuclei of such cells serve as controls in such preparations. In contrast, following treatment with ascorbic acid the osteoblasts round up and their cytoplasm becomes engorged with red staining material which we have shown to be ribose nucleic acid since it fails to stain with pyronin following digestion with malt diastase which contains a ribonuclease (10); even more convincing is the absence of coloration following digestion with crystalline ribonuclease[3] (11). To our knowledge this is the first unequivocal evidence that the cytoplasm of the osteoblast contains ribose nucleic acid.

LIPOID

Poorly differentiated cartilage cells contain lipoid, i.e., neutral fat, granules in their cytoplasm. There is no reduction of such lipoid in the cells of animals allowed to die in absolute scorbutus.

DISCUSSION

Since the discovery and synthesis of ascorbic acid in the early 1930's it has become increasingly difficult to know how to define scurvy. To be sure the now classic experiments of Wolbach and his collaborators (6–9) on the formation of collagen, osteoid, and dentine showed unequivocally that vitamin C, or ascorbic acid, has a specific effect on the deposition of these fibrous proteins. More recently, however, ascorbic acid has been shown to play a role in certain

[3] Worthington Biochemical Laboratory, Freehold,. N. J.

other physiological processes, for instance: the metabolism of the aromatic amino acids in both premature babies and in guinea pigs (12, 13); the secretion of the aqueous humor (14); a relationship to folic acid (15).

It would seem best to designate scurvy as that part of the overall picture of ascorbic acid deficiency which is characterized by a failure of certain specialized cells, i.e., fibroblasts, osteoblasts and odontoblasts to promote the deposition of their respective fibrous proteins: collagen, osteoid and dentine.

It would seem worth while to review briefly the morphological changes encountered in the development of skeletal scurvy. The effects of ascorbic acid deficiency are unique in that there is a complete dissociation of chondroblastic and osteoblastic activity. As in the normal sequences of endochondral bone formation, the cells forming the costal cartilage or epiphyseal plate reproduce and mature in normal fashion; inorganic materials are deposited between the rows of hypertrophic cells in the usual way. Here, however, the analogy to normal sequences stops. No osteoid, in which inorganic materials can be deposited, is formed on the spicules of calcified cartilagenous matrix, nor are any of these structures destroyed. As the cells of the epiphyseal or costal cartilage continue to proliferate, there appears a lattice of calcified cartilage matrix which is devoid of strengthening bone; as a result of mechanical forces such as muscle pull, particularly respiratory movements, fractures of this lattice occur, giving rise to the classical pathological picture of scurvy, i.e., *trümmerfeldzone*, *gerüstmark*, etc. The importance of these mechanical effects has been pointed out elsewhere (4). In scurvy there is no impairment of reproduction of mesenchymal cells as witnessed by the tremendously cellular *gerüstmark*. The spindle shaped cells which are present, however, are totally unable to influence the formation of fibers and bone matrix. Our understanding of the pathogenesis of scurvy is, of course, dependent on our understanding of the normal mechanisms operating in the formation of fibrous proteins of the connective tissue group. Of these processes we know virtually nothing. When scurvy heals there is a prompt return of function to the spindle shaped cells which round up and come to resemble osteoblasts again; in fact they become plumper than nornal osteoblasts.

The reduction in cytochrome oxidase activity as measured by the *nadi* reaction is of interest although the significance of this change is not at all clear. The possible relationship of this enzyme to the normal formation of bone matrix may only be surmised. The decrease observed is not due to inanition as studies in rats which have either been starved or whose growth has been interfered with in various ways indicate no change in cytochrome oxidase activity (16).

Whenever students of bone physiology come together it is certain that sooner or later the role of alkaline phosphatase will be brought up for discussion. It is now almost 30 years since Robert Robison (17) published his

provocative paper on "The Possible Significance of Hexosephosphoric Esters in Ossification" and described the enzyme which hydrolyzes such esters. Until recently all of the emphasis regarding the mode of action of this enzyme had been placed on its relationship to the calcification mechanism. Lately, however, since the role of the glycogenolytic cycle in the deposition of inorganic materials in cartilage has assumed a great deal of importance (18), the place of phosphatase in the calcification mechanism has become less clear than it was before. On the other hand this enzyme has come to assume more and more importance in connection with the elaboration of fibrous proteins.

In the newly formed collagenous fibers of healing wounds Fell and Danielli (19) noted intense phosphatase activity; the nuclei of regenerating fibroblasts were likewise darker than those of normal connective tissue cells. Bunting and White (20) also noted an increase in phosphatase activity in healing wounds; they drew the conclusion that fiber formation and phosphatsase were coincident but perhaps unrelated. On the other hand Robertson et al. (21), studying the formation of collagenous fibers in guinea pigs whose kidneys had been wrapped in cellophane, found no prominent increase in phosphatase activity and concluded that "the hypothesis—collagen fiber formation requires high levels of alkaline phosphatase—is untenable." This is the only negative report which has come to our attention. As far as bone is concerned, the fibers of normally developing membranous bone matrix are strongly positive when stained for alkaline phosphatase activity (22). So too, fibers of regenerating bone have been shown to exhibit positive reactions (23). One further possible relationship of phosphatase to the formation of fibrous proteins is found in certain lower forms, i.e. spiders and caterpillars (24). The glands forming the silk of these invertebrates are rich in phosphatase. The enzyme is found at exactly the same place where the first silk globules appear.

With respect to the relationship of ascorbic acid to phosphatase activity there is clear evidence that the two are related. Healing skin wounds in scorbutic animals show a virtual absence of fiber formation as well as phosphatase activity (25). Bourne (26) has shown that there is less phosphatase activity in regenerating bone from scorbutic animals than that from normal guinea pigs. More recently, Zorzoli and Nadel (27) have studied scorbutic bones both chemically and histochemically and find a significant decrease in alkaline phosphatase activity. These chemical studies confirm previously published observations on the phosphatase activity of bone by Gould and Shwachman (28).

From the above studies and the data derived from our own observations it would appear that there is a close relationship between the ascorbic acid content of tissues and alkaline phosphatase activity and that the latter may

be intimately concerned with the elaboration of fibrous proteins. The precise relationship is, of course, unknown. However, the enzyme need not be concerned solely with dephosphorylation but, as Gutman (18) has pointed out, may play a role in catalyzing the synthesis of phosphoric esters as well as acting as a transphosphorylase effecting the transfer of phosphorus from higher- to lower-energy phosphorus bonds. Furthermore alkaline phosphatase may act in the synthesis of nucleo-protein (29). One must further bear in mind the recent observations of Friedenwald and his associates (30) on substrate specific phosphatase enzyme systems and realize the probability that we should be more precise in any discussion of phosphatase in the future.

The presence of phosphatase activity and of glycogen and lack of any abnormality in metachromasia and in staining by the periodic acid-leucofucsin technique by the cartilage in absolute scorbutus is further evidence that the integrity of this tissue is unimpaired except as a result of mechanical factors which may produce profound changes in the zone of hypertrophic cells. We cannot confirm the observation of Meyer (36) that there is a loss of metachromasia in the cartilage of scorbutic guinea pigs.

The observations we have carried out indicate that the fine fibers which appear in the vicinity of osteoblasts following treatment, in addition to showing phosphatase activity, are metachromatic and argyrophylic (Figures 3, 4 and 5); the latter property, of course, places them in the category of reticulum fibers. A number of years ago Wolbach (7) noted the appearance of such argyrophilic fibers following therapy with vitamin C and their apparent aggregation to form collagen or osteoid bundles which, of course, do not stain with silver. Meyer (31) has suggested that reducing groups are present along the reticulum fiber which may be blocked or may have been removed in the formation of collagen. Osteoid is, of course, metachromatic (5), so that it is of interest though not unexpected that the fibrillary material which goes to make up the matrix of bone is also metachromatic. This tells us very little as to the combination of polysaccharide with the protein component of bone matrix, however.

The presence of ribose nucleic acid in the cytoplasm of the normal osteoblast is doubtless of some significance as will be shown below. We have described the appearance of the spindle shaped, impotent osteoblasts which make up in large part the *geriistmark* in absolute scorbutus. Such cells have very inconspicuous cytoplasm and when stained with hematoxlyn or pyronin there is very little basophilic material present. In contrast, following therapy with ascorbic acid such cells round up and the amount of basophilic cytoplasm increases considerably. This material has been shown by enzymatic digestion with crytalline ribonuclease to be ribose nucleic acid. The relationship of ribosenucleic acid to protein synthesis has assumed a great deal of importance in recent years. Caspersson (32) has formulated the principle that "all protein

synthesis needs the presence of nucleic acids." Of these the ribose nucleic acids are thought to be the most important. Both Caspersson (32) and Brachet (33) point out that those cells which have large amounts of ribose nucleic acid in their cytoplasm are the ones which have to do with the elaboration of proteins of one type or another. The osteoblast would, of course, fit into this category.

The absence of mucopolysaccharides and phosphatase activity in the *gerüstmark* and the apparent reduction in ribose nucleic acid in the osteoblast in absolute scorbutus and the prompt appearance of these constituents following the administration of ascorbic acid would certainly suggest that this vitamin is intimately related to the activity or formation of these materials. The precise mode of action of ascorbic acid and the biochemical transformations which occur are, at the present time, unknown.

One small part of the morphological picture of scurvy which has puzzled students of this disease for many years is the presence of an amorphous pink staining material about and between the clefts of the fractured spicules of calcified cartilagenous matrix. Aschoff and Koch (34) interpreted it as fibrin; Hojer (35) thought it was bone of an inferior type. Wolbach and Howe (6) felt it "had as its basis a product of the cells of the *gerüstmark*, probably liquid until added to by other materials from blood plasma or cartilage matrix resorption." Our histochemical studies have failed to elucidate the nature of this material further. It is not metachromatic nor particularly positive with the periodic acid technique. It stains blue with the Mallory stain. There is no phosphatase activity in it. It is not fibrin. Its exact chemical characteristics have yet to be determined.

SUMMARY

The following series of histochemical procedures have been applied to the bones of scorbutic guinea pigs either dying of absolute scurvy or following therapy for varying periods: cytochrome oxidase activity, phosphatase activity, as well as presence of glycogen, mucopolysaccharide, nucleoproteins, and lipoid. The results of these observations are presented and discussed.

BIBLIOGRAPHY

1. Follis, R. H., Jr., Some Histochemical Observations on Normal and Diseased Cartilage and Bone, Metabolic Interrelations, Trans. First Conf., Josiah Macy, Jr. Foundation, 1949, p. 27.
2. Follis, R. H., Jr., The Influence of Essential Nutrients and Hormones on Cartilage and Bone, Metabolic Interrelationships, Trans. Second Conf., Josiah Macy, Jr. Foundation, 1950, p. 221.
3. Follis, R. H., Jr., Studies on the Skeleton in Experimental Scurvy, Fed. Proc., 1950, **9:** 330.
4. Follis, R. H., Jr., Effect of Mechanical Force on the Skeletal Lesions in Acute Scurvy in Guinea Pigs, Arch. Path., 1943, **35:** 579.

5. Follis, R. H., Jr., and Berthrong, M., Histochemical Studies on Cartilage and Bone. I. The Normal Pattern, Bull. Johns Hopkins Hosp., 1949, **85:** 281.
6. Wolbach, S. B. and Howe, P. R., Intercellular Substances in Experimental Scorbutus, Arch. Path., 1926, **1:** 1.
7. Wolbach, S. B., Controlled Formation of Collagen and Reticulum. A Study of the Source of Intercellular Substance in Recovery from Experimental Scorbutus, Am. J. Path. Suppl., 1933, **9:** 689.
8. Menkin, V., Wolbach, S. B. and Menkin, M. F., Formation of Intercellular Substance by the Administration of Ascorbic Acid (Vitamin C) in Experimental Scorbutus, Am. J. Path. 1934, **10:** 569.
9. Boyle, P. E., Wolbach, S. B. and Bessey, O. A., Histopathology of Teeth of Guinea Pigs in Acute and Chronic Vitamin C Deficiency, J. Dent. Res., 1936, **15:** 331.
10. Lillie, R. D., On the Destruction of Cytoplasmic Basophilia (Ribonucleic Acid) and of the Metachromatic Basophilia of Cartilage by the Glycogen Splitting Enzyme Malt Diastase: A Histochemical Study, Anat. Rec., 1949, **103:** 611.
11. Brachet, J., La Detection Histochimique des Acids Pentosenucleiques, C. R. Soc. Biol. 1940, **133:** 88.
12. Levene, S. Z., Gordon, H. H., and Marples, E., A Defect in the Metabolism of Tyrosine and Phenylalanine in Premature Infants. II. Spontaneous Occurrence and Eradication by Vitamin C, J. Clin. Invest., 1941, **20:** 209.
13. Sealock, R. R. and Silberstein, H. E., The Excretion of Homogentisic Acid and Other Tyrosine Metabolites by the Vitamin C Deficient Guinea Pig, J. Biol. Chem., 1940, **135:** 251.
14. Friedenwald, J. S., Buschke, W., and Michel, H. O., Role of Ascorbic Acid (Vitamin C) in Secretion of Intraocular Fluid, Arch. Opth., 1943, **29:** 535.
15. Woodruff, C. W., Cherrington, M. E., Stockell, A. K., and Darby, W. J., The Effect of Pteroylglutamic Acid and Related Compounds Upon Tyrosine Metabolism in the Scorbutic Guinea Pig, J. Biol. Chem., 1949, **178:** 861.
16. Follis, R. H., Jr., Unpublished Observations.
17. Robison, R., The Possible Significance of Hexosephosphoric Esters in Ossification, Biochem. J., 1923, **17:** 286.
18. Gutman, A. B., and Yu, T. F., A Concept of the Role of Enzymes in Endochondral Calcification, Metabolic Interrelations, Trans. Second Conf., Josiah Macy, Jr. Foundation, 1950, p. 167.
19. Fell, H. B., and Danielli, The Enzymes of Healing Wounds. I. The Distribution of Alkaline Phosphomonoesterase in Experimental Wounds and Burns in the Rat, Brit. J. Exp. Path., 1943, **24:** 196.
20. Bunting, H. and White, R. F., Histochemical Studies of Skin Wounds in Normal and Scorbutic Guinea Pigs, Arch. Path., 1950, **49:** 590.
21. Robertson, W. V. B., Dunihue, F. W. and Novikoff, A. B., The Metabolism of Connective Tissue: Absence of Alkaline Phosphatase in Collagen Fibers During Formation, Brit. J. Exp. Path., 1950, **31:** 545.
22. Bevelander, G., and Johnson, P. L., A Histochemical Study of the Development of Membrane Bone, Anat. Rec., 1950, **108:** 1.
23. Bourne, G., The Distribution of Alkaline Phosphatase in Various Tissues, Quart. J. Exp. Physiol., 1944, **32:** 1.
24. Bradfield, J. R. G., Phosphatase Cytochemistry in Relation to Protein, Experimental Cell Research Supp. 1, 338, Academic Press, 1949.
25. Danielli, J. F., Fell, H. B. and Kodicek, D., The Enzymes of Healing Wounds.

II. The Effect of Different Degrees of Vitamin C Deficiency on the Phosphatase Activity in Experimental Wounds in the Guinea Pig, Brit. J. Exp. Path., 1945, **26:** 367.

26. Bourne, G. H., Alkaline Phosphatase Activity and Vitamin C Deficiency in Regeneration of Skull Bones, J. Anat., 1948, **82:** 81.
27. Zorzoli, A. and Nadel, E. M., A Histochemical and Chemical Study of the Alkaline Phosphatase Activity of Bone of Scorbutic Guinea Pigs, J. Nat. Cancer Inst., 1950, **6:** 1366.
28. Gould, B. S. and Shwachman, H., Bone and Tissue Phosphatase in Experimental Scurvy and Studies on the Source of Serum Phosphatase, Am. J. Physiol., 1942, **135:** 485.
29. Folley, B. S. J. and Greenbaum, A. L., Changes in the Arginase and Alkaline Phosphatase Contents of the Mammary Gland and Liver of the Rat During Pregnancy, Lactation and Mammary Involution, Biochem. J., 1947, **41:** 261.
30. Maengwyn-Davies, G. D. and Friedenwald, J. S., Histochemical Studies of Alkaline Phosphatases in the Tissues of the Rat Using Frozen Sections. I. Substrate Specificity of Enzymes Hydrolyzing Poly-glycol-mono-phosphate esters, Hexose-diphosphate, and β-naphthylphosphate, J. Cell. Comp. Physiol., 1950, **36:** 421.
31. Meyer, K. H., Rheumatoid Arthritis, Combined Staff Conference, Presbyterian Hospital, Am. J. Med., 1946, **1:** 675.
32. Caspersson, T., The Relationship between Nucleic Acid and Protein Synthesis, Symposium of the Society for Experimental Biology, Cambridge, 1947, p. 127.
33. Brachet, J., Nucleic Acids in the Cell and Embryo, Symposium of the Society for Experimental Biology, Cambridge, 1947, p. 207.
34. Aschoff, L., and Koch, W., Skorbut, Eine pathologisch-anatomische Studie, Jena, Gustav Fischer, 1919.
35. Höjer, J. A., Studies in Scurvy, Acta Pediat., Suppl., 1923, **3:** 8.
36. Meyer, A., Das Vorkommen der Chondroitin-schwefelsaure in skorbutischen Knorpel, Zeit. f. Vitaminforsch., 1944, **14:** 332.

ASSOCIATED MOVEMENTS BETWEEN LIMB AND RESPIRATORY MUSCLES AS A SEQUEL TO BRACHIAL PLEXUS BIRTH INJURY

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Received for publication April 27, 1951

Simultaneous contraction of muscles which do not normally act together, although supplied by the same nerve, has long been observed to follow injury to that nerve. Such associated movements have been recorded following regeneration of the facial nerve by Gowers (1888), of the oculomotor nerve by Bielschowsky (1935), and Ford and Woodhall (1938) pointed out that such phenomena may also be seen after regeneration of other peripheral and autonomic nerves. Little study has, however, been made of the associated movements after recovery from spinal root and plexus damage. Electromyographic records of such related activity between limb muscles after brachial birth palsy have been made by Leveuf et al. (1947). Likewise Sargent (1950) demonstrated action potentials, which were synchronous with respiration, in the biceps and forearm extensors in a patient eighteen years after birth injury. Clinical records of the frequency of this associated activity, however, do not appear to have been made. All adults available through the files of a general hospital, who had had brachial plexus damage at birth were therefore examined for evidence of such movements. The results in a series of eight unselected cases are recorded here.

METHODS OF EXAMINATION

The observations made in this series of cases are the result of clinical examination and fluoroscopic screening. Associated movements between limb and respiratory muscles were observed during inspiration. Conversely search was made for detectable effects of voluntary limb activity upon diaphragm and accessory muscles of respiration. The procedures were as follows:

1. *Examination of the limb at rest during quiet and deep inspiration.* The arm was held loosely by the side with the semi-pronated forearm supported at a right angle by the observer, and any associated movements in the limb muscles were seen or felt.

2. *Fluoroscopic screening of the diaphragm when the limb muscles contracted.* With the diaphragm in the position of expiratory apnoea the arm was flexed at the elbow against resistance. Any associated contraction of the diaphragm was seen on the fluorescent screen.

3. *Examination of the accessory muscles of respiration when limb muscles contracted.* Close observation of the accessory muscles of respiration was made during contraction of various limb muscles in turn. Muscular fixation of the joints was minimized by the observer holding the limb so that as many muscles as possible were relaxed, and all movements were made without resistance. For example, in contracting the flexors or extensors of the wrist, the upper arm was held to the side at the elbow and the semipronated forearm supported at a right angle by the observer. Similarly, in movements of the elbow, the upper arm was fixed by the observer and the forearm moved slowly from the position already mentioned.

In addition, note was made of simultaneous contractions of limb muscles, usually unassociated, which were both activated when an attempt was made to use one of them alone. In all of the above instances comparison was made with the normal limb or diaphragm.

OBSERVATIONS

The clinical picture of upper, lower, and whole arm types of brachial plexus palsy has been well described by Thomas (1905), Sever (1916), Ford (1944), and others. In this communication therefore, observations will be limited to those which appear to bear some relation to the conditions necessary for the appearance of the associated movements. For descriptive purposes these will be grouped under *Root and plexus*, *Cord*, and *Associated movements*.

Root and plexus. Of the eight cases of birth palsy, damage in four was limited to the upper roots of the brachial plexus; of the remaining four all showed weakness of muscles innervated by both the upper and lower roots, and in one only was the deficit more marked in the former. In the first group, referred to as the upper arm type, the paralysed muscles were the deltoid, biceps, brachialis, brachioradialis, supinator, and the long extensors of the wrist. The second group in this series was that variety known as the whole arm type. In none of these was the paralysis confined to the small muscles of the hand and the long flexors of the wrist and fingers as it is in the so-called lower arm type. In addition to the muscle wasting, weakness and reflex changes caused by damage to the roots, there was also in most cases evidence of some damage to the peripheral nerves which arise from the proximal parts of the plexus; in particular those to serratus anterior, rhomboids, and latissimus dorsi. Various degrees of recovery had taken place in these cases. On the whole it was greater in those patients of the upper arm group who had better finger movements, and hence more useful limbs.

Cord. Evidence for damage to the spinal cord was slight, and present in only three cases out of eight. Each had an "inverted" supinator reflex, and in one case there was also depression of the abdominal reflexes on the same side and hyperactivity of the ipsilateral knee and ankle reflexes. In none, however, was there an extensor plantar response.

Associated movements. Associated movement is considered to be present between two muscles or muscle groups when there is abnormal involuntary activity of any degree in one, accompanying contraction of the other. The movements seen in the cases here described were not of sufficient magnitude to move the larger joints although the brachioradialis stood out under the skin in some cases, fingers flexed in others, and the forearm rotated in two. In most cases, however, the contractions were small, localised, and often flickering in nature. A few were so slight as to be invisible, but they were readily palpable.

In six of the unselected series of eight cases, there was unequivocal asso-

ciated movement in the limb muscles on inspiration. The abnormal movements were seen only with deep inspiration and even then they were not sufficient to disturb the patients, the majority being unaware of them until attention was drawn to them. The two upper arm cases showing this unusual activity had contractions in biceps, brachioradialis, and deltoid. Of the four remaining cases, which were of the whole arm type of palsy, contraction of those muscles alone was seen only in that case which had suffered more damage to the upper roots. In the other three, in which the lower roots were more involved, contractions in forearm muscles were seen in addition to similar activity in those three muscles already mentioned. In one case, flexors of the ring and index fingers, and triceps were involved; in the second, pronator teres, index flexor, and thumb abductor were active; in the third, thumb and ring finger contracted. It was found, therefore, that associated movements between limb and respiratory muscles were seen during inspiration only in muscles which had been weak as a result of damage to their nerve supply.

The effect of contraction of the limb muscles on the diaphragm was studied by fluoroscopy in five of the six cases showing the abnormal movements just described. In three, a small contraction of the diaphragm was clearly seen on the fluorescent screen when the elbow was flexed against resistance. One of these had a moderately severe upper arm type of lesion, and the other two had had whole arm palsies—in one the damage to the upper roots being equal to, and in the other greater than, that to the lower roots. Therefore all the cases showing this associated movement had sustained injury to the upper part of the plexus.

Clinical inspection of the accessory muscles of respiration during activity of various limb muscles revealed only one case in which localised contraction occurred in the pectoralis major with flexion of the elbow and also of the fingers. This was observed in the case with the most severe permanent damage.

Various associated movements between muscles of different groups in the limb were noted when one joint was moved at a time, and descriptions of these can be found in the case histories in the appendix.

DISCUSSION

An explanation of the abnormal muscular contractions recorded above requires an understanding of the nature of birth injury. The results of damage to the plexus must have been known since forceful intervention has been used in difficult labour, but a true conception of the mode of injury has only been obtained by analysis of the conditions under which it occurred and by experimental studies. The first case reported in the medical literature is attributed to Smellie (1764) and he considered the palsy was due to prolonged pressure on the arms while the baby was in the pelvis. All later workers interested in the subject noticed that the palsies occurred during difficult labour; and Clark

et al. (1905) point out that Duchenne believed that traction on the roots was the cause, but Erb's theory, which incriminated the pressure of the accoucheur's fingers on the roots, held general acceptance for a long time. Fieux (1897) supported the traction theory, and furthermore by dissecting new born cadavers he showed that displacement of the head away from the shoulder stretched the upper two roots of the plexus much more than the lower ones. The differential effect of such traction was emphasized by finding much wider separation of cut ends of the upper roots when all were severed, and the head then flexed laterally. He proved that, in an undissected cadaver, if this movement were continued beyond a point where the roots were merely taut, a point was reached where a snap was heard, and dissection showed the upper two roots were torn and the others intact. Finally he produced incomplete paralysis in the forelimb of a rabbit by forcibly pulling the head to one side with the limb fixed. The particular vulnerability of the upper two roots which he thus amply demonstrated was confirmed by later workers. Boyer (1911) showed on the cadaver that traction on the arm in a plane at right angles to the middle line of the body would injure the seventh cervical root, and Sever (1916) was able to damage the lowest two roots of the plexus by strong abduction and extension of the arm. He also added the observation that damage to the upper roots was much more likely to occur when the clavicle was fractured, since this allowed the whole weight of the arm to bear directly on the plexus. In addition to the root damage caused by traction some of the peripheral nerves are also torn, in particular the suprascapular nerve and the nerves to rhomboids and serratus anterior. The phrenic nerve may also be involved during difficult delivery, causing irregular respiratory rate and rhythm, and cyanosis. All five such cases recorded by Tyson and Bowman (1933) had had forceps applied to hasten delivery and in all except one, brachial palsy was also produced. Positions of the arm and head in relation to each other and to the body, such as have been described above are frequently found during certain complicated manoeuvres made during prolonged and difficult labour, and the likelihood of injury therefrom may well be increased by other factors such as the large size of the baby, loss of muscular tone and resistance during anaesthesia.

Pathological examination of the plexus post mortem has been described by Prout (see Clark et al. (1905)), Sever (1916), and others. Macroscopically the picture varied according to the extent and character of the force producing the lesion. The severity of the original haemorrhage and exudate determined the amount of fibrous tissue and scarring found in the late stages. Concerning the nerve fibres themselves, whether they were roots, trunks, or cords, all degrees of damage from complete severance to haemorrhage beneath the perineurium were found. The junction of the fifth and sixth cervical roots—Erb's point—was a common site of such damage. A frequent microscopic finding was tearing of the vessels in the nerve sheath, with bleeding

into or beneath it. In others this haemorrhage extended into the nerve itself, rupturing the fibres. The extent of such extravasation and its ultimate organisation will determine the amount of functional recovery. Such are the conditions after injury to the plexus, but recovery is not merely a question of some nerve fibres being torn and not uniting, and others simply rejoining distal sheaths of Schwann, for Cajal (1928) has shown experimentally that reunion of the central and peripheral ends of regenerating fibres occurs in considerable disorder. The individuality of the axon is lost and the distribution of its branches and connections becomes much disturbed. The fine fibres which arise from the distal end of a cut axis cylinder exhibit a topographical indifference, such that they may come to innervate any of the branches of the cut nerve. Cajal demonstrated experimentally in the rabbit that after section of the peroneal and tibial branches of the sciatic nerve, fibres arising from the cut surface of the central portion of each nerve innervated the peripheral stumps of both branches. Further, by section of a cat's peripheral nerve in three successive regions it was shown that any obstruction to the regenerating fibrils by small scars and the like, caused them to lose their orientation and in many cases to bifurcate and innervate two sheaths of Schwann.

The above outline shows how abnormal neural connections may occur between two muscles or muscle groups which previously were not actively related. Similar histological evidence of a new anatomical pattern following nerve injury has been given by Howe et al. (1937) in their experimentally produced facial palsies in monkeys, recovery from which was accompanied by associated movements. An explanation of the abnormal simultaneous activity between the limb and respiratory muscles is therefore possible on a structural basis. The respiratory muscles in question are the diaphragm, and the accessory muscles of respiration, namely—trapezius, sternomastoid, serratus anterior, the pectoralis, latissimus dorsi, and the scalene muscles. All of the latter group except the first two named are innervated by nerves having their origin in the fifth to eighth cervical and first thoracic roots which form the brachial plexus and supply the limb muscles also. The part of the diaphragm which arises from the septum transversum is innervated by the phrenic nerve which itself receives a rootlet from the fifth cervical root. Injury to the fifth root proximal to the origin of this rootlet may permit the central regenerating fibres, which originally went to the arm, to descend into the phrenic nerve and innervate the diaphragm; thereby contraction of the limb muscles will cause the diaphragm to descend. Similarly, contraction of accessory respiratory muscles may occur during limb movements when the plexus itself is damaged at a point where innervating fibres run close together. The converse, namely contraction of arm muscles during inspiration, may be produced by injury at either of the sites mentioned.

As it was found by clinical observation in this series of cases that only deep

inspiration produced this abnormal activity it was impossible to be certain whether the re-innervation of the limb muscles had come from phrenic nerve fibres or from nerves supplying the accessory respiratory muscles. The reverse procedures demonstrated that aberrant fibres might come from either one or from both. Whichever was responsible in any individual case, in six of the eight patients examined here, clinical observation was adequate to detect such associated movement, suggesting that the phenomenon is much more frequent than has been thought hitherto.

SUMMARY

Eight unselected patients who had suffered brachial plexus birth palsy were examined at intervals of from nine to twenty-three years after injury. Associated movements between muscles of respiration and partially paralysed limb muscles were found in six of them, as shown by contraction of the latter during deep inspiration. To demonstrate that such abnormal activity might occur as a result of damage either to the phrenic nerve or to the nerve supply to the accessory respiratory muscles, the effect of use of the limb muscles on those of respiration was studied. Fluoroscopy in five of the six cases demonstrated diaphragmatic contractions in three, when the elbow was flexed. In one case contraction of the limb muscles produced associated activity in one of the superficially placed accessory respiratory muscles. The explanation for these functional connections following injury is discussed and their probable frequency emphasized.

I wish to express my gratitude to Dr. F. R. Ford and Dr. J. W. Magladery for their helpful advice and my thanks are also due to members of the Radiological Department for assistance with screening. The work was carried out while the author was holding a Nuffield Foundation Fellowship.

REFERENCES

Bielschowsky, A.: Lectures on motor anomalies of the eyes. II. Arch. Ophthal. **13:** 33 (1935).

Boyer, G. F.: The complete histopathological examination of the nervous system of an unusual case of obstetrical paralysis 41 years after birth, and a review of the pathology. Proc. roy. Soc. Med. **5:** Sect. Neurol. 31 (1911–12).

Cajal, S. R.: Degeneration and Regeneration of the Nervous System. Translated and edited by R. M. May, London. Oxford Univ. Press. (1928) pp. 310 and 312.

Clark, L. P., Taylor, A. S. and Prout, T. P.: A Study on Brachial Birth Palsy. Amer. J. Med. Sci. **130:** 670 (1905).

Fieux, G.: De la Pathogénie des paralysies brachiales chez le nouveau-né. Paralysies obstetricales. Ann. Gynec. Obstet. **47:** 52 (1897).

Ford, F. R.: Diseases of the Nervous System in Infancy, Childhood and Adolescence. Springfield. Thomas. 2nd Edition (1948).

Ford, F. R. and Woodhall, B.: Phenomena due to misdirection of regenerating fibres in cranial, spinal, and autonomic nerves. Arch. Surg. **38:** 480 (1938).

GOWERS, W. R.: A Manual of Diseases of the Nervous System. London. Churchill. Vol. II. 1st Edition (1888) p. 224.

HOWE, H. A., TOWER, S. S. AND DUEL, A. B.: Facial tic in relation to injury of the facial nerve. Arch. Neurol. Psychiat., **38:** 1190 (1937).

LEVEUF, J., LECOEUR, P., LEFEBRVE, J. AND LERIQUE, J.: Contractions paradoxales des muscle au cours des paralysies obstetricales du plexus brachial (troubles de la synergie). Rev. Orthopéd. **33:** 305 (1947).

SARGENT, F.: Birth injury of the left arm showing crossed reinnervation from the phrenic nerve into nerves supplying biceps, extensor digitorum communis and other muscles. Proc. roy. Soc. Med. **43:** 252 (1950).

SEVER, J. W.: Obstetric Paralysis. Amer. J. Dis. Child. **12:** 541 (1916).

SMELLIE, W.: A Collection of Cases and Observations in Midwifery. London. D. Wilson and T. Durham. Vol. II. P. 506 (1764).

THOMAS, J. J.: Two cases of bilateral birth paralysis of the lower arm type. Boston Med. Surg. J. **153:** 431 (1905).

TYSON, R. M. AND BOWMAN, J. W.: Paralysis of the diaphragm in the newborn. Amer. J. Dis. Child. **46:** 30 (1933).

APPENDIX

Case 1, N. S., female, 18 years (J. H. H. No. 79129). Spontaneous vertex delivery at term; 9 pound baby; no cyanosis. Bruising over the shoulder and upper chest with left upper arm type of palsy. Gradual return of function in fifteen months.

There was limitation of external rotation of the shoulder joint. The arm was slightly thinner and shorter than the normal one and the hand was of less bulk. Slight weakness was found in the serratus anterior, pectoralis major, infraspinatus, deltoid, biceps, brachioradialis, supinator, pronator teres, and the long and short extensors of the thumb. No sensory loss was found. The biceps reflex was absent; the supinator reflex was replaced by brisk finger flexion; the knee and ankle reflexes on the side of the weak arm were brisker than those on the right and the ipsilateral abdominal reflexes were depressed. Both plantar responses were flexor.

Contractions within the brachioradialis, biceps, and deltoid were seen with deep inspiration. A sudden inspiratory sniff produced localised activity in the deltoid. Flexion of the elbow was accompanied by contractions in the biceps, deltoid, brachioradialis, and infraspinatous. No movement of the diaphragm was seen with flexion of the arm.

Case 2, D. D., female, 18 years (C. H. S. No. 5726).[1] Vertex presentation at term; forceps delivery; 7¾ pound baby; right upper arm type of palsy. There was slow recovery and capsulotomy of the shoulder joint was performed at 12½ years of age.

Examination revealed wasting of the arm, with 1 inch of shortening in the upper arm and 1½ inches in the forearm; marked limitation of abduction and external rotation of the shoulder joint was present. Weakness was moderate in the rhomboids, biceps, triceps, brachioradialis, and supinator; marked in subscapularis, latissimus dorsi, and deltoid, the last named being very wasted. Defective localisation over the fifth cervical dermatome was the only sensory abnormality. All deep reflexes in the arm were absent.

Deep inspiration was accompanied by visible contractions in the deltoid and biceps, and palpable contractions in the brachioradialis. Flexion of the elbow caused activity in the deltoid. Descent of the diaphragm accompanied flexion of the arm.

[1] This patient had been under the care of Dr. R. E. Lenhard at the Children's Hospital School, Baltimore.

Case 3, J. M., male, 18 years (J. H. H. No. 78083). Vertex presentation at term; forceps delivery; 10¼ pound baby; left whole arm palsy. No cyanosis at birth but present with otitis media at five months of age. Very slow recovery of function.

On examination there was 2½ inches of shortening in the upper arm, ½ inch in the forearm, and the left side of the chest was not so well developed. Marked limitation of external rotation of the shoulder, and slight limitation of elbow extension were found. Wasting was present in the deltoid, biceps, and brachioradialis with a moderate degree of weakness. This latter was slight in the rhomboids, moderate in the extensors of the forearm, fingers, and thumb, and severe in the spinati. No contraction was felt in the latissimus dorsi. There was impairment of all forms of superficial sensation over the shoulder region down to the deltoid insertion. The biceps reflex was absent, the supinator reflex was replaced by finger flexion and the triceps reflex was brisk; those of the legs were symmetrical.

Case 4, J. I., male, 20 years (J. H. H. No. 69838). Vertex presentation at term; forceps delivery; 10½ pound baby; no cyanosis; left whole arm paralysis. Very slow recovery with only slight power in any muscle reported at one year. Tenotomy of the subscapularis and teres major was performed at 6½ years of age; six months later a rotatory osteotomy of the left radius was carried out, open reduction thereof being necessary nine months later.

Examination revealed limitation of abduction and external rotation of the shoulder, and extension of the elbow. The whole limb was wasted, with 1½ inches of shortening in the upper arm and 2¼ inches in the forearm. Weakness was slight in the rhomboids, latissimus dorsi, biceps, triceps, brachioradialis, pronator teres, and extensor carpi radialis; severe in the spinati, deltoid, supinator, and the remaining extensors of the forearm; very severe in the flexors of the forearm, and small muscles of the hand and thumb. Pain sense was intact but some light contacts were missed on the hand; postural sense was preserved in all fingers but vibration sense was impaired below the elbow. Localisation of light touch was inaccurate over the outer surface of the upper arm, extensor surface of the forearm and dorsum of the hand. All deep reflexes in the arm were absent.

Deep inspiration was accompanied by contractions in the biceps, triceps, deltoid, supinator, and flexors of the ring and index fingers. A brief expiratory snort caused localised activity in the triceps. Flexion of the elbow was accompanied by contractions in the deltoid, which were also seen, together with similar activity in triceps and infraspinatus, during wrist extension. These two muscles were also active with finger flexion, extension of which was accompanied by activity of the latter muscle alone.

Case 5, J. C., male, 23 years (J. H. H. No. 167184). Instrumental delivery at term; 9½ pound baby; right whole arm palsy. Poor subsequent recovery, so plexus was explored at 2½ years of age. A "bulbous mass", taken to be a neuroma, was exposed at the junction of the fifth and sixth cervical roots and adhesions were so dense that little could be done. He wore a splint until four years of age and not long after discarding it he fractured the lower end of the humerus on that side, since which time he has noticed limitation of elbow extension and also loss of sensation in the forearm. When twelve years old, further damage to this arm resulted in slipping of the upper humeral epiphysis; after recovery from this, a sliding graft of the wrist was carried out.

There was generalised wasting of the limb with 2½ inches of shortening in the upper arm and 3¾ inches in the forearm. Considerable limitation of passive movement of the shoulder and elbow was found, pronation—supination was reduced to about 15 degrees and the wrist was ankylosed. Weakness was moderate in the rhomboids, biceps, brachioradialis, pectoralis major, and latissimus dorsi; extreme in the spinati, deltoid, triceps and pronator teres. Power of thumb flexors and abductors was moderately reduced, but there was scarcely any voluntary movement in its extensor or in the small hand muscles. Radial finger flexors

were stronger than ulnar; active extension was seen in the middle finger only. Sensory loss was a mixture of root and peripheral involvement; loss of cutaneous sense over the fifth cervical dermatome, defective localisation over the sixth, and loss again over the ulnar border of the forearm and the whole hand. All deep reflexes in the arm were absent.

Deep inspiration caused contractions within the deltoid, biceps, and brachioradialis, with slight pronation of the forearm, thumb abduction, and index flexion. Elbow flexion was accompanied by similar contractions in thumb and index flexors, deltoid, and pectoralis major. Such activity was seen in the last two named, together with contraction of pronator teres with finger flexion. Downward movement of the diaphragm accompanied arm flexion.

Case 6, E. C., male, 9 years (C. H. S. No. 7287).[2] Vertex presentation; delivery hastened by traction on the arm; 12½ pound baby; reported as "black" at birth; right whole arm palsy. Recovery was poor after a year and at eight years of age an osteotomy of the mid-radius was performed to correct a supination deformity.

The whole arm was found wasted, with ¼ inch shortening in the upper arm and 1¼ inches in the forearm. Passive shoulder abduction and external rotation, and elbow extension were all limited. There was a right sided Horner's syndrome with a normally pigmented iris. Marked wasting in shoulder girdle, forearm, and hand muscles; moderate weakness in rhomboids, spinati, pectoralis major, latissimus dorsi, biceps, and triceps; severe in brachioradialis. Forearm flexors resisted slightly against gravity but extensors only acted with this factor eliminated. Small hand muscles all extremely weak. No localised abnormality of sensation was found. Biceps and triceps reflexes were absent and the supinator reflex was replaced by finger flexion. The leg reflexes were normal.

A small localised deltoid contraction was seen when a sudden inspiratory sniff was made, and deep inspiration caused activity in that muscle as well as in brachioradialis and ring finger flexor; occasional twitches being noticed in the thumb flexor also. Elbow flexion was associated with contractions in deltoid.

Case 7, J. B., male, 20 years (J. H. H. No. 68149). Spontaneous vertex delivery; 10 pound baby; no cyanosis; left upper arm palsy. The upper arm was shorter by 1 inch, the forearm by 1¼ inches and that hand was smaller. Severe limitation of the shoulder abduction and external rotation, and slight limitation of elbow extension, were present. Weakness was slight in the latissimus dorsi, biceps, triceps, brachioradialis, extensor carpi radialis longus, and extensor digitorum, but more marked in rhomboids and deltoid, this latter being markedly wasted. There was slight impairment to all forms of sensory testing over the fifth cervical dermatome. Biceps reflex was sluggish, and supinator and triceps reflexes were absent.

No associated movements, other than contractions in deltoid with elbow flexion, were seen.

Case 8, L. P., male, 14 years (J. H. H. No. 99441). Spontaneous delivery at term; 9½ pound baby; left upper arm palsy. Good recovery by 18 months of age.

The forearm was ½ inch shorter, but no wasting was visible. Weakness was minimal in brachioradialis and extensor carpi radialis longus, and only just detectable in the thumb extensors. Localisation of touch was inaccurate over the fifth cervical dermatome but all deep reflexes were symmetrical, and no associated movements were seen.

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[2] This patient had been under the care of Dr. George E. Bennett at the Children's Hospital School, Baltimore.

THE USE OF TERRAMYCIN IN ROCKY MOUNTAIN SPOTTED FEVER*

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Received for publication May 4, 1951

Terramycin is another streptomyces-derived antibiotic whose broad range of activity encompasses a number of microbial agents of disease (1). In addition to its numerous antibacterial powers, it has been shown to possess potent anti-rickettsial properties when tested in laboratory animals and the chick embryo (2, 3). Moreover, it has been shown to be effective in the treatment of patients with tsutsugamushi disease (2) and murine typhus fever (4). Its mode of action is believed to be rickettsiostatic.

Previously, results obtained in two patients who received terramycin for Rocky Mountain spotted fever have been reported (4). This paper summarizes the findings in those two patients and in five additional patients with this rickettsial disease.

METHOD OF STUDY

Selection of cases

During the period from May to July 1950, seven patients with Rocky Mountain spotted fever were referred to the University Hospital. Four of these patients were children aged 3, 4, 7 and 12 years whereas three were adults aged 27, 34 and 81 years.

A. *Clinical Criteria.* Utilizing criteria previously described (5), patients selected for treatment presented typical features of Rocky Mountain spotted fever. All patients had headache and rash typical of this disease as well as sustained fever from the onset of illness.

B. *Laboratory Criteria.* Confirmation of the clinical diagnosis of Rocky Mountain spotted fever was sought in each instance through: 1) inoculation of patients' blood into guinea pigs and 2) examination of serial specimens of blood for detection of Proteus OX 19 and OX 2 agglutinins and specific complement-fixing antibodies. The details of these procedures have been outlined previously (5).

Method of administration and dosage of terramycin

Terramycin† was in the form of 250 mg. capsules. Little difficulty was experienced with oral administration except for vomiting of the initial dose in three instances.

* This study was supported by a grant from the Charles Pfizer Company, New York, N. Y.

† Terramycin used in this study was provided by Charles Pfizer and Company.

Terramycin was administered in initial doses of approximately 50 mg./kilo body weight. In adults, approximately 3.0 gm. were given initially followed by doses of 1.0 gm. every eight hours. Children received approximately two-thirds the adult dose. Therapy was continued until the temperature reached normal levels provided defervescence was accompanied by apparent clinical improvement. The disease in one patient (case 7) was of such severity that undue delay in clinical improvement made it judicious to supplement treatment with intravenous chloramphenicol.

Determination of terramycin serum levels was performed during the course of therapy (6).

Clinical results

The mean day of disease on which treatment was instituted in this series was 6.4. In five cases improvement of the patients' symptoms was observed

TABLE I

Tabulation of Results in Patients with Rocky Mountain Spotted Fever Treated with Terramycin

CASE	AGE	SEX	DAY OF DISEASE STARTED	TOTAL DOSE (GM.)	DURATION TEMP. AFTER TREATMENT (DAYS)	TOTAL HOSPITAL (DAYS)	LABORATORY DATA				
							Rickettsemia	Weil-Felix		Comp. Fix.	
								Acute	Conv.	Acute	Conv.
1.	3	F	4	6.1	2.5	7	Positive	0	40	80	160
2.	4	F	10	2.75	2.2	11	Positive	80	2560	0	160
3.	12	F	4	7.75	2.0	6	Positive	0	0	0	160
4.	7	M	7	6.0	1.5	8	Positive	160	1280	40	160
5.	27	M	8	16.0	3.0	13	Positive	0	80	0	10
6.	81	F	4	9.0	3.5	15	Negative	0	5120	0	0
7.	34	M	8	8.0*	10.5	20	Positive	1280	5120	160	640

* Treatment changed to intravenous chloramphenicol on tenth day of disease.

within the first 24 hours. On the second day of treatment, there was striking abatement of such symptoms as headache, weakness and mental dullness. In an elderly woman of 81 (case 6), amelioration of the clinical symptoms was not noticed until the second treatment day followed by marked improvement one day later. Case 7, seriously ill on the eighth day of disease showed no improvement after two days of treatment with terramycin. Actually, there was progression of the toxic delirium and skin rash and no significant alteration of the febrile course. In all patients with the exception of case 7, the eruption did not spread following initiation of treatment, and after 48 hours it had markedly receded. On the third day, six of the seven cases were convalescent showing interest in their surroundings, increased vigor and general symptomatic improvement.

The rapidity with which temperatures receded lends further confirmatory evidence of the favorable effect exerted by terramycin on the course of Rocky

Mountain spotted fever. (In this study normal temperatures are defined as rectal temperatures under 100° or oral temperatures under 99°.) Irrespective of the age of the patient, height of the preceding fever or day of the disease, terramycin therapy was followed in six of the seven cases by defervescence within 90 hours after the initial dose. See table I. Indeed, the average duration of fever in these six cases was considerably less, approximately 2.5 days (60 hours). Patient #7 is not included in the computation of these average values because an additional antibiotic was utilized in the management of his disease. After initiating terramycin therapy, the duration of fever in case 7 was 10.5 days. It is most probable, however, that without antibiotic treatment this patient would have succumbed to the toxemia of the disease. In no instance after defervescence was there a return of fever or symptoms of sufficient magnitude to indicate a relapse.

Convalescence in these patients proceeded uneventfully. In three cases administration of terramycin was attended by mild gastro-intestinal disturbances (e.g. nausea, vomiting and diarrhea). These side effects did not necessitate cessation of therapy. Repeated urinalyses showed no abnormality. There were no significant alterations in hemoglobin levels or red and white blood cell counts attributable to the antibiotic.

Laboratory results

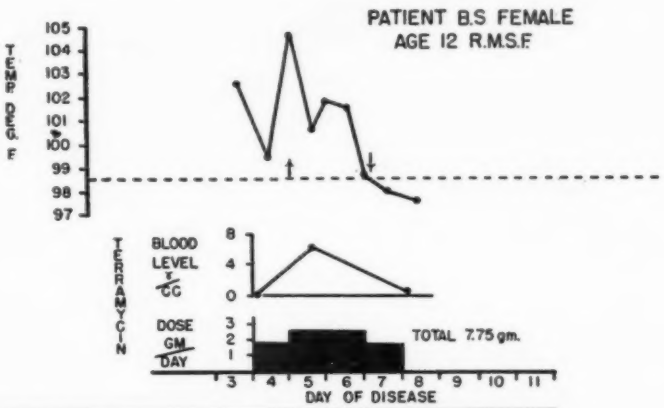
A. *Effect on Rickettsemia.* In six of seven cases, rickettsemia was demonstrated by inoculation of blood into guinea pigs. In three of these cases, attempts were made to re-isolate rickettsia 24 and 48 hours after therapy was instituted. None of these attempts was successful but the data are insufficient to conclude that rickettsia disappear rapidly after initiating terramycin treatment.

B. *Effect upon Serologic Response.* In six of the seven cases an increasing titer of agglutinins for Proteus OX 19 or OX 2 was observed although two of these six failed to develop titers higher than 1:80. The serum of one patient examined as late as the sixteenth day of disease demonstrated no agglutinins for the Proteus group. In six patients, a raising titer of complement fixing antibodies was demonstrated whereas one patient (case 6) failed to show this antibody. However, the latest specimen available on this patient was drawn on the eighteenth day of disease.

C. *Blood Levels of Terramycin.* In all patients, serum concentrations of terramycin were determined (6). Serum specimens were examined at random with relation to time of drug administration. Nevertheless, in five patients bioassay of two or more specimens during the course of treatment revealed levels ranging from 0–12 gamma/cc., mean 5.4 gamma/cc. In two patients, cases 6 and 7, measurable levels were not obtained which may have been a factor accounting for the delay of clinical response.

ILLUSTRATIVE CASE REPORTS

Case 3, This twelve year old white girl was admitted to the hospital on the third day of an illness characterized by severe frontal headache, nausea, vomiting and high fever. Contact with ticks had been frequent during the two weeks prior to onset of disease. In the afternoon of the third febrile day a faint, pink, macular, ill-defined rash was detected on the forearms, legs, palms, soles and trunk. The temperature on the fourth day of disease was 104° and all symptoms seemed to be aggravated. At this time, there was a moderate amount of meningismus. Terramycin treatment was instituted with a dose of 1.75 gm. orally and subsequent doses of 0.75 gm. every eight hours. Slight nausea accompanied the administration of the large initial dose. After twenty-four hours of therapy meningismus



RICKETTSIEMIA		+								
COMPLEMENT FIXATION				0						1:160(90th day)
WEIL FELIX	OX 19			0			0			
	OX2			0			0			
W.B.C. (THOUSANDS)		3.6								

FIG. 1 (case 3). Graphic record of a 12 year old girl with Rocky Mountain spotted fever

had disappeared, headache had subsided and the child's irritability had diminished. Within forty-eight hours, the patient was comfortable and the rash and signs of meningeal irritation had vanished. The patient became and remained afebrile approximately forty-eight hours after terramycin was first administered. A total of 7.75 gm. had been given. Pertinent clinical and laboratory findings are represented in figure #1 below.

Case 4, The patient, a seven year old boy, entered the hospital on the seventh day of an illness manifested by malaise, anorexia, intense frontal headache and fever. On the third day of disease a pink, macular rash appeared on the extremities and trunk. On hospitalization, the patient had a petechial rash over the pelvis, soles, forearms, legs and trunk as well as hepatomegaly and splenomegaly. The child was oriented but irritable. On the seventh febrile day an initial dose of 1.5 gm. of terramycin was given followed by doses of 0.75 gm. every eight hours the second treatment day and 0.5 gm. every eight hours the third day for a total of 6.0 gm. Within twenty-four hours after instituting therapy, the rash was less intense, no new lesions were detectable and headache was ameliorated. The following day

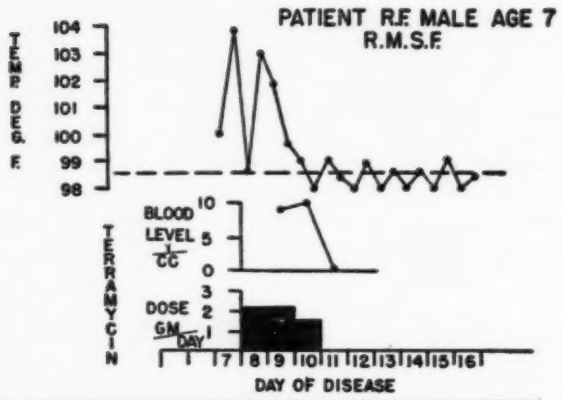


FIG. 2 (case 4). Response in a boy with Rocky Mountain spotted fever treated with terramycin.

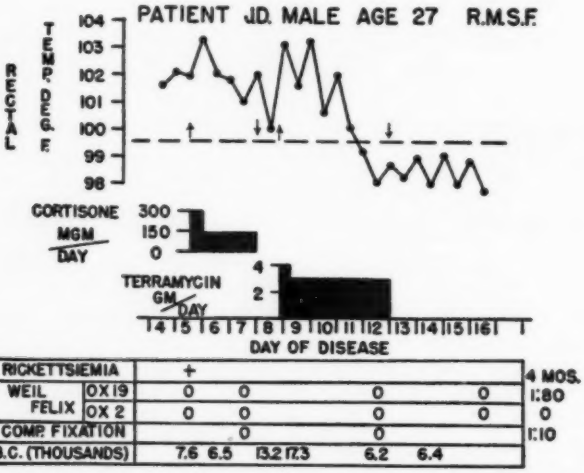
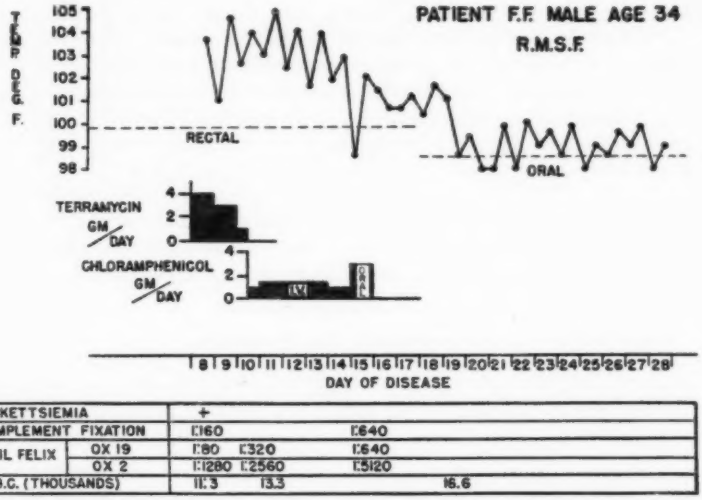


FIG. 3 (case 5). Course of Rocky Mountain spotted fever in a patient treated with cortisone and terramycin.

the patient was decidedly improved; he was alert, more cheerful and ate well. After thirty-eight hours of treatment, the temperature reached and remained at normal levels. Convalescence progressed satisfactorily and the patient was discharged after ten days of hospitalization. Pertinent findings are graphically represented in the figure below.

Case 5, A twenty-seven year old farmer was hospitalized on the fourth day of disease complaining of severe frontal headache, chills and fever. Ten days prior to the onset of illness a tick had been removed from the abdomen. On the third day of illness, a pink eruption was noted on the arms and legs. When admitted to the hospital the patient was mentally clear but complained of intense frontal headache which was aggravated by coughing or moving about. The rash was maculo-papular in character extending over the extremities, axillae and flanks. On the fifth day of disease, the patient received 300 mg. of cortisone acetate intramuscularly and subsequent doses of 150 mg. daily for two additional days. There was no significant improvement with this therapy. In fact, headache persisted and the rash extended. It may be noted in figure #3 that there was a tendency to lowering of the tem-



RICKETTSIEMIA		+
COMPLEMENT FIXATION		1:160 1:640
WEIL FELIX	OX 19	1:80 1:320 1:640
	OX 2	1:1280 1:2560 1:5120
W.B.C. (THOUSANDS)		11.3 13.3 16.6

FIG. 4 (case 7). Graphic record of a severely ill patient with Rocky Mountain spotted fever. Treatment with terramycin and chloramphenicol.

perature while cortisone was being administered. On the evening of the eighth day the patient received an initial dose of 3.0 gm. of terramycin and subsequent doses of 1.0 gm. every eight hours for three days. Within thirty-six hours after beginning this treatment, the headache disappeared and there was striking increase in strength, vigor and appetite. Temperature reached normal levels in seventy-two hours after beginning the antibiotic treatment. The further clinical course was uneventful. Pertinent findings are presented in figure #3.

Case 7, The patient, a 34 year old man, was hospitalized on approximately the eighth day of an illness characterized by fever, headache and a body eruption. When first examined the patient was quite toxic and complained of intense headache. Speech was slurred and indistinct. The conjunctivae were markedly injected. A diffuse maculo-papular rash, petechial in character, was spread over the entire body including the face. The scrotum showed several areas of ecchymotic hemorrhage. The blood pressure was 90/60. Rales were heard in the lungs and there was moderate splenomegaly and hepatomegaly. The patient's condition was regarded as serious. Without delay, an initial 3.0 gm. dose of terramycin was

given followed by doses of 1.0 gm. every eight hours. Treatment was supplemented with transfusions of saline, glucose and blood plasma. After forty-eight hours the patient was still incoherent, restless, incontinent and dehydrated despite energetic supportive therapy. Skin lesions progressed; the scrotal lesions became gangrenous. Blood pressure was 110/65. The abdomen was markedly distended making administration of antibiotic difficult. It is noteworthy that two blood specimens obtained at this time showed no measurable terramycin. Beginning at noon of the tenth day of disease, when the patient's condition was considered desperate, intravenous chloramphenicol therapy was initiated. Chloramphenicol was given in doses of 500 mg. intravenously every six hours. Intravenous infusions of saline and glucose and transfusions of whole blood were continued. Within thirty-six hours (twelfth day of disease) although his temperature remained elevated, the patient's mental status was somewhat clarified and his abdominal distension was decreased. On the fourteenth day the patient was markedly improved from all aspects. The temperature remained at a definitely lower level. From this point onward, convalescence proceeded favorably.

It cannot be surmised that this patient represents a terramycin treatment failure nor can it be categorically denied. Indeed, it is conceivable that failure to respond was due to faulty absorption from the atonic gastro-intestinal tract which resulted in an insufficient therapeutic concentration of antibiotic in the blood and tissues. Moreover, it is well known that patients with Rocky Mountain spotted fever treated late in the course of their illness respond much less rapidly than earlier cases and that energetic support of the circulatory system is vital for eventual recovery. Intravenous chloramphenicol seemed to tip the balance in favor of recovery. The pertinent findings are graphically represented in figure #4 below.

DISCUSSION AND SUMMARY

Previous experience with Rocky Mountain spotted fever in Maryland has shown that this rickettsial disease has a fatality rate of approximately 21 per cent. Furthermore, the average duration of the acute febrile course has been fifteen and eighteen days among pediatric and adult survivors respectively. In analyzing the data compiled on patients with this disease in the University Hospital from 1930–1945, there has been little variation in severity of the illnss from year to year from point of view of mortality rates, duration of illness and length of hospitalization. The seven patients reported in this paper are representative of this rickettsiosis as it occurs in Maryland. We have been unable to incorporate a control group of cases in this study for obvious reasons.

The remarkable effectiveness of chloramphenicol (5) and aureomycin (8) in treatment of Rocky Mountain spotted fever is now of general knowledge. With either of these broad spectrum antibiotics, mortality from this disease has been practically eradicated and the clinical manifestations of the illness have been arrested in about two to three days. It has been sufficiently emphasized in other publications that sole reliance upon the antibiotic in seriously ill patients late in the course of their disease is inadequate. Energetic treatment utilizing transfusions of saline, glucose, plasma and whole blood is vital for support of the embarrassed circulatory system.

From the data presented it appears that terramycin, an antibiotic substance

possessing a broad range of activity, must share in this effectiveness. In six of seven patients treated there was striking abatement of the clinical symptoms within forty-eight hours and absence of obvious signs of illness within seventy-two hours. Moreover, the average duration of fever after beginning the antibiotic in this group was sixty hours which compares favorably with the results obtained with aureomycin and chloramphenicol. One adult patient, classified as critically ill with Rocky Mountain spotted fever, failed to show significant improvement after two days of treatment with oral terramycin. The failure of this patient to attain a satisfactory level of antibiotic in the blood was thought to have been caused by faulty absorption from the gastro-intestinal tract. Administration of intravenous chloramphenicol results in a prompt high concentration of antibiotic and recovery ensued. Conceivably, a comparable form of terramycin would have been equally effective. Terramycin hydrochloride suitable for intravenous use is now available.

The question of proper dosage of terramycin cannot be settled from results obtained in this small number of patients. An initial loading dose calculated on the basis of 50–75 mg. per kilo is recommended followed by doses of 1.0 gm. every eight hours for adults. Children should receive approximately two-thirds the adult dose. Antibiotic therapy may be safely terminated after the temperature has reached normal levels provided the clinical manifestations of the disease have been arrested at that time. Terramycin treatment was attended by certain symptoms indicative of mild gastro-intestinal irritation (e.g. nausea, vomiting and mild diarrhea.) Therapy was not discontinued because of these side effects.

CONCLUSIONS

Six of seven patients with Rocky Mountain spotted fever experienced rapid recoveries after treatment with terramycin. One patient failed to show improvement presumably because of his failure to attain a therapeutic blood concentration after oral administration.

BIBLIOGRAPHY

1. Finlay, A. C.; Hobby, G. L.; P'an, S. Y.; Regna, P. P.; Routien, J. B.; Seeley, D. B.; Shell, G. M.; Sobin, B. A.; Solomons, I. A.; Vinson, J. W. and Kane, J. H. Terramycin, a new antibiotic. Science **85**: 111, 1950.
2. Smadel, J. E.; Jackson, E. B. and Ley, H. L. Jr. Terramycin as a rickettsiostatic agent and its usefulness in patients with scrub typhus. Ann. New York Aca. Sc. **53**: 375–384, 1950.
3. Snyder, J. C.; Fagan, R.; Wells, E. B.; Wick, H. C. and Miller, J. C. Experimental studies on the antirickettsial properties of terramycin. Ann. New York Aca. Sc. **53**: 362–374, 1950.
4. Bauer, R. E.; Parker, R. T.; Hall, H. E.; Benson, J. F.; Joslin, B. S.; Hightower, J. A.; Snyder, M. J.; Venable, S. J. and Woodward, T. E. Clinical and experi-

mental observations with terramycin in certain rickettsial and bacterial infections Ann. New York Aca. Sc. **53**: 395–406, 1950.

5. Pincoffs, M. C.; Guy, E. G.; Lister, L. M.; Woodward, T. E. and Smadel, J. E. The treatment of Rocky Mountain spotted fever with chloromycetin. Ann. Int. Med. **29**: 656–663, 1948.
6. Parker, R. T. Unpublished data.
7. Parker, R. T.; Bauer, R. E.; Lister, L. M.; Woodward, T. E. and Hall, H. E. Further experience in the treatment of Rocky Mountain spotted fever with chloramphenicol. Am. Jour. Med. **9**: 308–314, 1950.
8. Ross, S.; Schoenbach, E. B.; Burke, F. G.; Bryer, M. S.; Rice, E. C. and Washington, J. A. Aureomycin therapy of Rocky Mountain spotted fever. J. A. M. A. **138**: 1213, 1948.

MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

In Association with the Alpha Omega Alpha Fraternity

Monday, April 9, 1951

The first order of business was the election of officers for the coming year. Dr. Allan L. Grafflin was elected President and Dr. Richard H. Follis, Jr. was re-elected Secretary.

Dr. Fisher: Will the meeting come to order.

We have a very interesting program here. We are continuing this year, as we have for the past several years, in having this last meeting in association with the Alpha Omega Alpha Fraternity.

Dr. Carol G. Johnson will take charge of the remainder of the program.

Dr. Johnson: In the absence of the President of the Alpha Omega Alpha Chapter, Dr. Rainey Williams, it is my privilege to announce the new members of our Chapter from the Fourth Year Class. (Names were read by Dr. Johnson.)

The first paper will be presented by Mr. Carl Kupfer of the Third Year Class.

HISTOCHEMISTRY OF MUSCLE CHOLINESTERASE IN THE RAT FETUS AND AFTER MOTOR NERVE SECTION

The earliest appearance of significant amounts of cholinesterase (ChE) in the skeletal muscle of the forelimb of the rat as determined manometrically with a series of homogenates was noted on approximately the sixteenth day of fetal life. The enzyme was found histochemically, using the method of Koelle and Friedenwald (Proc. Soc. Exp. Biol. and Med. **70**: 617–622) to be localized at that time chiefly on the surfaces of special muscle nuclei. These special muscle nuclei were found in successive stages to aggregate at one locus within each muscle cell at approximately the same level as in the adjacent fibers. The configurations so formed are first clearly seen at birth (21–22 days) and are essentially indistinguishable from the adult pattern by the fifth day post partum.

Thirty-nine days after motor nerve section, the end plate configuration associated with ChE activity decreased about three-fourths in area, the greater portion of this decrease occurring within the first sixteen days.

It appears from the present study that the ChE activity is chiefly associated with the specialized muscle nuclei of the sole plate.

Dr. Johnson: Mr. Kupfer's paper is now open for discussion. Are there any comments or discussion? If not, the next paper will be presented by Miss Elizabeth D. Hay* of the Third Year Class.

THE ROLE OF EPITHELIUM IN AMPHIBIAN LIMB REGENERATION STUDIED BY HETEROPLOID EPIDERMAL TRANSPLANTS

The wound epithelium plays a vital role in the regeneration of amphibian limbs, yet little is known about its important functions. Rose (1948, J. Exp. Zool., **108**: 337) has suggested that the wound epidermis dedifferentiates to supply a portion of the cells of the young re-

*Henry Strong Denison Scholar in Anatomy for 1949–1951.

generation blastema. The purpose of the present experiment is to test this hypothesis by tracing the epidermis with heteroploid transplants.

Haploid, diploid and triploid *Rana pipiens* tadpoles were used as donors and hosts in various combinations. Tail epidermis characterized by a different degree of ploidy from that of the host was transplanted to the thigh of the right hindlimb of 50 tadpoles, the left hindlimb serving as a control. Both limbs were previously stripped of skin and amputated through the ankle. Regenerates were fixed in different stages of growth, sectioned, and stained with Heidenhain's hematoxylin. In practice, the most reliable criterion for the identification of the transplant cells proved to be the maximum number of nucleoli in the nucleus, which varies directly with the number of sets of chromosomes in the cell.

In the first stage of the regeneration of these limbs, the transplanted epidermis is found migrating toward the wound surface of the amputated limb. Its basement membrane is left behind on the thigh. In the second stage of regeneration, the transplant epidermis is found in the thick epithelial mound at the tip of the limb in 3 out of 8 cases, remaining proximal to the tip or dying in the other cases. In the third stage, when the epithelial mound disappears and the early blastema appears, one limb shows numerous large mesenchyma-like triploid and tetraploid cells inside the blastema which have come from the triploid-tetraploid epidermal transplant which this diploid limb received. The control limb is completely diploid. Inasmuch as 5 of the 9 limbs fixed in stage 3 have fused nucleoli in the blastema cells, so that no reliable criterion of ploidy is present, this positive result is one out of 4 cases. Regarding its significance, since the epithelial mound is assumed to be the source of the transforming cells but only contained transplant epidermis in 3 out of 8 cases, it is not reasonable to expect a large incidence of positive blastemata in stage 3 in as small a series as this one. In stage 4, proliferation and differentiation begin. In 16 limbs at this stage, the transplant cells are not present in the epithelium or in the undifferentiated blastema. In the differentiating tissues it is not possible to identify transplant cells because the nucleoli cannot be recognized.

In summary, the present experiment shows that epidermis can supply a portion of the cells of the young regeneration blastema. Because the method is not adequate at present for those tissues which do not possess clear-cut nucleoli, it is not known whether or not these transformed epidermal cells in the blastema redifferentiate into the cartilage and muscle of the new limb.

Dr. Johnson: Miss Hay's paper is open for discussion. Dr. Grafflin.

Dr. Grafflin: We have been fortunate enough to have Miss Hay in the Department of Anatomy during the development of this work, and from her we have learned a great deal. The problem is an extremely difficult and complex one, and was in a highly unsatisfactory state when she entered it. As Miss Hay has explained, it is still in an unsatisfactory state, but in my judgment she has given us the most critical experimental series to date. I should like to congratulate her upon her experimental strategy and her excellent technic.

Dr. Howard: Where did you get this transplant tissue? Did that come from a younger animal?

Miss Hay: I didn't go into that. I used triploid epithelium. In a tadpole, triploid animals can be produced by heat-treating the eggs right after they have been fertilized. This causes the egg to keep the second polar body, which gives it an extra set of chromosomes. Such eggs develop into animals that are completely triploid. All their cells are triploid and they do not

revert to the diploid type. Epidermis stripped from these triploid tadpoles is used for the transplant. We got one tetraploid animal by chance and that gave the positive result I mentioned. At Princeton they are breeding triploids and getting tetraploids and pentaploids. Such cells would be much better for cell-tracing than triploid, because they are so large.

Dr. Howard: The animal from which you derived the transplant isn't any younger?

Miss Hay: The donors and the host tadpoles were the same age.

Dr. Mirick: I have another question. In your diagram, stage 4, you seem to show that the actual transplant material was still there, whereas the cells that had migrated down to the end were disintegrated. Did the graft maintain its integrity?

Miss Hay: Well, it was not actually that. The black line shown in the diagrams is the basement membrane of the transplant. This basement membrane can be still recognized for a week or two after stage 4 begins and then it finally disappears. But it isn't actually cellular material. The fact that it remains on the thigh for so long is one point against the argument that the transplant cells disintegrated. We really don't know what happened to the transplant cells after stage 3.

Dr. Johnson: Are there any further questions on this paper?
The next paper will be presented by Mr. Thomas R. Hendrix.

STUDIES ON CRYPTOCOCCUS POLYSACCHARIDE

Dubos and Avery have isolated a specific enzyme from a soil bacillus capable of hydrolysing the type specific polysaccharide of the capsule of type III pneumococcus. This enzyme can protect mice against one million lethal doses of type III pneumococcus and they showed that the protective value was proportional to the ability to hydrolyse the capsular material.

Since the capsule of Cryptococcus or Torula is polysaccharide it was decided to apply the approach of Dubos and Avery to the Cryptococcus.

Soil samples were planted in inorganic media containing 0.02% Cryptococcus capsular polysaccharide (prepared according to the method of Kligman) as the sole source of carbon. In thirteen days the Molisch reaction (a nonspecific colorimetric test for polysaccharides) became negative for polysaccharide and on second transfer a negative Molisch was produced in eight days.

Pure culture of the various organism isolated failed to produce a negative Molisch.

The most likely explanation was that the organism sought catalysed the first step only, which did not remove polysaccharide but merely split the complex polysaccharide into smaller units which still gave a positive Molisch reaction.

Since there was no specific test for the capsular polysaccharide, hydrolysis was tested by measuring changes in the flow time of the polysaccharide. Enzyme preparations were prepared by lysing cultures of the isolated organism which had been grown on the polysaccharide media. Controls were prepared by heating the lysed bacterial suspension to boiling for thirty minutes. This did not change the flow time of the suspensions. On unheated lysed suspension after incubation eighteen hours at 37° with 1.25% capsular polysaccharide reduced the flow time in an Ostwald viscometer from 189 seconds of the heated control to 143 seconds. The mixture without polysaccharide had a flow time of 103 seconds.

In summary, a mixed culture of soil bacteria was able to so alter the Cryptococcus capsular polysaccharide in a simple culture media, in which it was the sole source of carbon, that the Molisch reaction became negative in thirteen days and in eight days on second transfer.

One of these organisms when grown on the simple polysaccharide media contained an active principle which is destroyed by heat and which can reduce the viscosity of Cryptococcus capsular polysaccharide.

Dr. Johnson: Mr. Hendrix's paper is open for discussion. Dr. Mirick.

Dr. Mirick: This represents a tremendous amount of work. It is obviously the first stage and what may turn out to be a useful clinical tool. I say that because the enzyme which Dubos isolated, and Mr. Hendrix mentioned, is active, not only *in vitro*, but actually cured experimental animals from pneumococcal infection. It has been tested on rabbits, mice and likewise monkeys, and never used clinically in human beings only because more useful, that is less specific therapeutic agents became available at the time that the enzyme work was completed—first the sulfonamides and, of course, the other antibiotics coming on later. In the case of Cryptococcus infections there are, at present, no other antimicrobial or chemotherapeutic agents which are proved to be effective and it is quite possible that an enzyme would be of use in treating such infections.

A great deal still remains to be solved. First: i_t is not known for sure that the Cryptococcus is contained in one serological type. Adapt *i*ve enzymes of the type Mr. Hendrix has described are notoriously of great specificity and any difference at all in the types might limit the general effectiveness of the enzyme even though it may hydrolize the capsule of one strain. Second: there is as yet, no proof that virulence of Cryptococcus depends upon the presence of its capsule. By analogy to pneumococcus, hemophilus and other bacteria this is probably true, but I am not acquainted with any studies concerning Cryptococcus without capsule. Third: we do not know, or Mr. Hendrix does not know yet, whether the reaction will work in the animal body, or whether the enzyme may be toxic.

Of course, there are limitations to using this method of treatment, because such an enzyme would probably be antigenic and would thereby tend on injection into an animal body to stimulate antibodies which would, in time, counteract its activity. If effective in a fairly short time it might have clinical applicability.

I hope that in the next year Mr. Hendrix will continue with this problem and perhaps come up with some of the answers we are all interested in having.

Dr. Fisher: I think there is a very good chance that this will have clinical application. As Mr. Hendrix and Dr. Mirick have said, this particular infection, so far, has been resistant to every type of therapy. It runs all sorts of clinical courses. In some cases the infection is localized—then it may be drained, or reached locally by an enzyme or some antibiotic agent. In others, the meninges only are involved, and infection of the meninges has been fatal as far as I know in practically all cases, though it may go on and run a chronic course for quite a long time. Another type is the generalized infection of the blood stream in which many organs are involved.

Now, the characteristic feature of the Cryptococcus is that in the tissues at least,—that is in the body fluids of the patient infected with it, and in mouse experiments which have been done a large capsule is formed, and in every virulent strain that has been studied this capsule is produced. In localized infections the capsular substance is always a feature of it. Consequently, if that can be attacked, there is a very good chance that the host can take care of the infecting organism itself. Of course, as Dr. Mirick said, enzymes may be changed. Such therapy would be very difficult to carry out in many of these cases, and it is not known whether the enzyme could get to the infected areas in large enough concentration to sterilize them or help the patient get over them. I think these adaptive enzymes are very interesting to study in every type of situation such as this.

In the pneumococcus, as has been said, when the capsule is gotten rid of, the pneumococcus itself is not killed, but can be phagocytized much more easily, and it may be that the same thing holds true for this type of organism.

Dr. R. Austrian: Cryptococcus provides another example of the general importance of surface components to the pathogenicity of microorganisms. We find that such substances play an essential role in the virulence of many bacterial species, this being true not only of polysaccharide but also of protein antigens. The importance of this outer covering is demonstrated clearly by the fact that its removal by an enzyme renders the invading organism susceptible in a number of instances to the cellular defense mechanisms of the host. It is important to recall, however, that the bacterium or fungus is not altered genetically by exposure to the enzyme directed against its surface component and, when removed from contact with it, will regain rapidly its normal structure. The suppression of virulence achieved by such enzymatic activity is therefore transitory, and infection may only be overcome with the aid of host defense mechanisms during the period of structural alteration of the invader.

Dr. Bang: May I ask Mr. Hendrix if this enzyme has an effect on the Cryptococcus that you can see with a microscope?

Mr. Hendrix: We tried one short experiment in which the soil bacillus and Cryptococcus were incubated together. Nothing convincing could be seen, but the culture was only incubated for one week.

Dr. Johnson: Are there any further questions or comments?

The next paper will be presented by Mr. Andrew M. Nemeth of the Second Year Class.

GLYCOGENESIS IN THE FETAL GUINEA PIG LIVER

Claude Bernard first made the observation that glycogen appears very early in the maternal placenta but relatively late in the fetal liver. The onset of glycogen storage in the developing vertebrate liver is a tardy and abrupt event which takes place after the elapse of 65% of the total gestation period in the guinea pig. Dalton transplanted liver cells from a preglycogen stage chick embryo to the chorioallantois of a chick embryo whose liver contained glycogen. He found that the transplanted parasite cells failed to elaborate glycogen any earlier than would have occurred had they been left in the donor animal. Insull incubated liver slices in a glucose medium to determine if the difference between preglycogen and glycogen stages persisted in the absence of such extrinsic factors as carbohydrate demand and hormonal influence. He found synthesis of glycogen in liver which normally contains it, no synthesis in preglycogen stages. Thus we may conclude that the onset of glycogen storage is dependent upon factors in the hepatic cells themselves and is not dependent upon the environment of these cells.

In view of these findings it was decided to investigate the possibility that one of the enzymes involved in glycogen synthesis limits by its low activity the formation of glycogen until late in fetal life. It was found that the activities of phosphorylase and phosphoglucomutase are at the adult level throughout fetal life and long before the onset of glycogen storage.

The presence of polysaccharide primer was established in the preglycogen stage fetal liver by incubating homogenates of such livers with glucose-1-phosphate and barium chloride and demonstrating the synthesis of polysaccharide by the formation of a purple color on the addition of Lugol's solution. Homogenates of fetal liver which contained glycogen *in vivo* also synthesized *in vitro* a polysaccharide which formed a purple color on addition of

Lugol's solution. Adult liver homogenates synthesized a polysaccharide giving a claret color on addition of Lugol's solution. This indicates that the activity of the branching enzyme is less in the fetal glycogen-containing liver than in the adult liver.

In the preglycogen stage fetal liver priming polysaccharide is below chemically detectable levels but phosphorylase and phosphoglucomutase activities are at the adult level. We tentatively conclude that the activity of the branching enzyme is the limiting factor and when it reaches an adequate level glycogen storage becomes detectable. Quantitative studies on hexokinase and branching enzyme activities are now in progress.

Dr. Johnson: Mr. Nemeth's paper is now open for discussion. Dr. Flexner.

Dr. Flexner: Just by way of letting you know how long it takes to get some places, I might remark that Mr. Insull, Dr. Insull now, first undertook this problem five or six years ago as a first-year student, and worked on it the four years he was here. He laid a firm foundation for Mr. Nemeth's work of the past year. I should like to congratulate Mr. Nemeth on his contributions, both technical and conceptual. He has added a great deal, it seems to me, to our insight into the problem.

Dr. Johnson: Are there any other comments or discussion?

The final paper will be given by Mr. H. David Mosier, Jr. of the Third Year Class.

RESPONSES OF THE RAT ADRENAL TO A HIGH SALT DIET

Studies with wild and domestic Norway rats have revealed striking differences in their water and electrolyte balance. Thirty per cent of captive wild Norway rats have three to four times the water intake of the average domestic rat. Adrenalectomized domestic rats are easily maintained by administration of desoxycorticosterone acetate (DOCA) and most will survive if given access to a salt solution; whereas DOCA is less effective in maintaining adrenalectomized wild rats and salt solution fails almost completely.

In a recent experiment we studied the adrenals of wild and domestic Norway rats on normal diets and diets containing up to seventy per cent sodium chloride to determine whether the adrenals would reflect anatomically or histochemically the differences mentioned. The salt diets and tap water were given ad lib. Rats of both strains on diets containing up to twenty-five per cent of sodium chloride had nearly the same sodium intake and retained body weight and appearances of health until sacrificed seventy to ninety days later. Diets with greater percentages of salt were lethal to rats of both strains in ten to forty days. High salt intake caused an atrophy of the glomerulosa zone of the adrenals in both strains. This atrophy consisted of a decrease in the cell volume and consequent narrowing of the glomerulosa zone. Frozen sections stained with Sudan IV revealed that high salt intake caused a complete disappearance of sudanophilic lipid in the glomerulosa zone of the domestic rat. The wild rat, on the other hand, always contained an appreciable amount of sudanophilic lipid in the glomerulosa zone. The significance of this difference is not known at this time.

Dr. Johnson: Mr. Mosier's paper is open for discussion. Dr. Howard.

Dr. Howard: This is an interesting observation of Mr. Mosier's. We are accustomed to the atrophy of glands following administration of their products to the organism, as, for example, atrophy of the thyroid when thyroxin is given. Current evidence supports the assumption that sodium retaining hormone is secreted by the glomerulosa and this outer

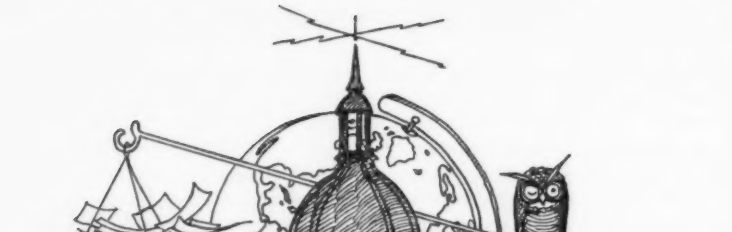
zone of the adrenal is said to have selectively atrophied after desoxycorticosterone administration. In Mr. Mosier's experiments, however, it appears that this zone has shrunk from a high sodium intake, as though when its hormone's work was done for it, the gland atrophied. It is as if the blood sugar were kept low (by some means other than insulin), the pancreatic islets would atrophy. Removal of the need for the hormone, that is, removal of stress for making the hormone, results in atrophy. In other instances cited, thyroxin and cortisone, it is pretty well proven that the pituitary tropic hormones have been tamponaded rather than the effect being directly on the thyroid and adrenal glands themselves.

In Mr. Mosier's experiments with the wild rats, he finds lipoid material remaining in the glomerulosa even after high salt ingestion. The inference is that these zones are still actively producing sodium retaining hormones. One wonders if this may not be merely a matter of degree, a stage in atrophy which may be functionally insufficient and yet not anatomically atrophied. This could be tested by suddenly depriving such animals of all salt and administering potassium and seeing if they developed signs of adrenal insufficiency.

Mr. Mosier: We haven't tried that type of experiment, Dr. Howard. It will be interesting to see in future experiments what effect is produced in the adrenals by changing the diet from high salt back to normal or to a diet containing abnormal amounts of another electrolyte.

Dr. Grafflin: It could not possibly mean as much to these students to present these papers as it does to the staff to hear them. A valid test of a good medical school is whether, through the years, a reasonable number of medical students engage with enthusiasm in more or less independent investigative work. So long as a group of our students continues to conduct research of the high caliber we have heard presented tonight, I cannot get unduly worried about the present or the future of this institution. I have thoroughly enjoyed the speakers this evening, and I should like to congratulate them all.

Dr. Johnson: In closing, Alpha Omega Alpha would like to thank the Johns Hopkins Medical Society for affording us the opportunity to present these reports of student research.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Introduction to Surgery. By VIRGINIA KNEELAND FRANTZ and HAROLD DORTIC HARVEY. 233 pp. $2.75. *Oxford University Press, New York.*

The presentation of the elements of the broad field of surgery in a manner at once intelligent, attractive, comprehensive, and well-organized to a group of second year medical students is a formidable yet important task. It is too frequently true that instruction at this level may be poor in perspective, or haphazard in preparation, overlooking the absence of the clinical background of the student. Yet it is at this time that the first interests that lead to choice of a specialty are often aroused.

This monograph, written in keeping with the "philosophy of the inquiring mind," is complementary to the introductory (second year) course in surgery at the College of Physicians and Surgeons. Drs. Frantz and Harvey have continued to conduct this course following the retirement of their provocative and stimulating predecessor, Dr. William Cogswell Clarke. Stress is placed upon the essential principles of injury, inflammation, and repair with especial reference to surgical lesions.

After a short introduction and history, injury and repair are discussed in some detail. The application of these principles in each group of tissues and lesions is clearly demonstrated. Short discussions on contact with the patient, surgical technique, anesthesia, plastic and experimental surgery, and recent advances conclude the small volume.

As a working handbook for second year students, and as a guide to their instructors this book is superb. It accomplishes its purposes in a manner worthy of highest praise.

J. C. H.

Paul Ehrlich. By MARTHA MARQUARDT. 255 pp. $3.50. *Henry Schuman, Inc., New York.*

In these days of imminent human catastrophe, it would be heartening if we could in some way make a reckoning of the alleviation of human suffering brought about by the chemotherapy of infectious disease. No doubt the number saved would outweigh those lost in previous wars. Yet the man who started this chemotherapy has not previously had any real

biography. Now comes his former secretary to write a personal, deeply moving and also a fairly complete scientific story of his development. Her first little book written in 1924, some nine years after Ehrlich died, was later destroyed by the Nazis.

One wants to know "How did such a man work? How did such a genius exert his influence?" He might himself answer. "Geduld, Geschick, Geld and Gluck" (patience, ability, money and luck) or with advice like that given to his assistant, "Dear Dr. Franke: remember—much experimenting—the utmost exactitude in experiments—the absolute minimum of hypothesis—much work—no guess work—few publications—wissen Sie—verstehen Sie." But this biography shows how much more than that there was to it—how an intuitive imagination and a deep sense of humanity's suffering was combined with a dominating personality. Assistants must carry out the experiments indicated on the little notes which were continually left in their laboratories. The research of others in his institutes was not merely encouraged and facilitated; it was constantly directed, sometimes producing bad feelings if not results.

This biography is long overdue. Every physician should acquire some image of the little bespectacled man with his many strong cigars, his absent-minded professorial air, his constant interest in the outcome of the treatment of every case of syphilis. Thus, one may finally see him as he listed the results of treatment and the batches of salvarsan on the door of his cluttered laboratory so that the pencil marks reached from top to bottom. Then perhaps those who come so many years later will gain new courage so that some day scientific research on human ailments may again become the property of all men, of all creeds and of all nationalities.

F. B. B.

Physical Diagnosis, 4th edition. By RALPH H. MAJOR. 446 pp. $6.50. *W. B. Saunders Co., Philadelphia, Pa.*

The 4th edition of Dr. Major's highly popular and widely used text carries on in the same tradition of excellence established in earlier editions. Numerous minor alterations of the text represent definite improvements, but the basic organization and content remain unchanged. Consequently, the technique of history taking and recording is still to be found in the last chapter although the author frequently emphasizes the fundamental importance of this subject in the interpretation of physical findings. Also, as in previous editions, abnormal findings in diseases of the lungs are presented to the student before the normal findings of the cardio-vascular system are discussed.

There are but few changes in illustration aside from phonograms, electrocardiograms and pulse tracings. For the most part, the illustrations are graphic and serve their purpose well. However, in some instances, such as "rose spots" and "spider naevi," black and white photography is inadequate and the space occupied by such illustrations could be employed for other purposes.

The most valuable portions of the text are clearly those chapters concerning the heart and lungs, but it is also here that one encounters some of the chief shortcomings. The student will find additional and improved phonograms, but must be alert for a few incorrect reference numbers and labels. At times, there is room for confusion in the description of abnormal findings in certain disease states, and one would prefer to see more effort made to analyze the symptoms and signs presented by the major disease syndromes in terms of dynamic pathologic physiology. However, in spite of the foregoing criticisms, the author continues to have no contemporary equal in the presentation of significant historical backgrounds, in the description of classical symptoms and signs of major disease syndromes, and in the firm, uncompromising adherence to the fundamental scientific aspects of physical diagnosis.

This text, therefore, continues to be of real value to all students and to most graduate physicians.

R. L. McL.

Fundamentals of Clinical Fluoroscopy: With Essentials of Roentgen Interpretation. By CHARLES B. STORCH. 196 pp. $6.75. *Grune & Stratton, New York.*

There has long been a definite need for a simply written, concise book on clinical fluoroscopy. The modern physician shows intense interest in learning the principles of fluoroscopy. He understands the value of fluoroscopy as a diagnostic tool in general practice, internal medicine, cardiology, and gastroenterology. Dr. Storch's book fulfills this purpose admirably. The standard radiologic literature is much too scattered and technical to serve such a general need. Since the book is an outgrowth of a series of lectures by the author it retains the flavor of a lucid teaching style.

The book is divided into 4 chapters. Chapter 1 presents the basic concepts including the elements of producing a fluoroscopic image, x-ray protection, and dark adaptation. In his discussion of adaptation the author, for the most part, uses the correct term "dark adaptation" and only occasionally slips into the erroneous term "accomodation" which is so widely used. The second chapter discusses fluoroscopy of the chest from the point of view of technique, normal anatomy, and pathology. The third chapter deals with fluoroscopy of the heart, and considerable text and illustrative material is utilized to explain the right and left oblique projections of the cardiovascular system. Chapter 4 begins the section on the gastrointestinal tract and concerns itself with fluoroscopy of the pharynx, hypopharynx, and esophagus. This chapter and the subsequent chapters on the stomach and colon are perhaps somewhat too specialized for the general practitioner and quite elementary for the radiologist or gastroenterologist. On the whole, the book is well illustrated and easy to read. Its chief virtue lies in the lucid description of the conduct of the chest fluoroscopic examination with particular reference to the heart. The entire book is 196 pages and definitely is a welcome aid to medical students, house officers, and those wishing to study the elements of fluoroscopic examinations.

D. M. G.

Functional Anatomy of the Limbs and Back. A Text for Students of Physical Therapy and Others Interested in the Locomotor Apparatus. By W. HENRY HOLLINSHEAD. 341 pp. $6.00. *W. B. Saunders Co., Philadelphia, Pa.*

The subtitle of this book expresses its prime purpose, which is to provide a useful account of what the Germans have so aptly termed "Bewegungsapparate," more particularly of the limbs and the back, for those who are interested in the functions of muscles and in bodily movements. Since the book is aimed at those who possess little knowledge of biology and none of human anatomy, it begins with a brief, elementary introduction to the organization of the body (comprising sections on anatomical terminology, the tissues of the body, and organs and organ systems) and ends with a short account of head, neck, thorax, and abdomen.

These first and last sections are well done and should be useful to non-medical students of all sorts who are interested in the human body. Physiological data are included, thus elevating the account above the realm of deadhouse anatomy.

The three main sections of the book, on the upper limb, the back, and the lower limb, respectively, go into much greater detail. Not only muscles, bones and joints, but also nerves, fascias and blood vessels are included. The physiology of movement is stressed throughout. The illustrations are well chosen and well executed. The treatment is such that these sections

can serve, as the author notes, as a ready reference or review for advanced students or practitioners, for it is stripped of anatomical minutiae and limited to the essentials of the subject. At the same time, details of importance are not neglected.

Dr. Hollinshead has produced a useful book, for intelligent functional expositions of human anatomy are rare. But it remains a question in the mind of the reviewer whether the main portion, dealing with the limbs and the back, can be adequately digested by those without benefit of some previous or accompanying dissection of the human body. This is not due to any limitation of the author, who has selected his material well and who has written clearly, but rather to the nature of the subject itself.

W. L. S., JR.

Klinische Chemie und Mikroskopie. By LOTHAR HALLMANN. 594 pp. Ganzleinen DM 24.60 ($5.85). *Georg Thieme Verlag, Stuttgart, Germany.*

This book, in its sixth edition, represents an ambitious undertaking to describe laboratory procedures used in many fields of medicine. The size of the book is relatively small, and the abundance of fine print makes much of the reading difficult. Nevertheless, the organization and presentation of the material are excellent. The 166 figures and 7 colored plates are clear and concise. Scientific words of Latin and Greek origin are given sufficient preference over those of strictly Germanic origin, so that the reader with moderate knowledge of the German language should experience relatively little difficulty in interpretation.

Descriptions of laboratory apparatus and elementary techniques are written in great detail. There are excellent, if occasionally didactic, discussions of the patho-physiological background and of the principles involved in the various procedures. Yet relatively little emphasis is placed upon difficulties encountered in final clinical interpretation. Differentiation between routine laboratory procedures and special tests to be used only upon specific indication is frequently not clear.

The sections dealing with qualitative and semi-quantitative examinations of the various body fluids and in particular that concerning the urine are excellent. A general criticism of the book concerns the lack of specific references to the original work on various tests. In the sections on hematological and quantitative chemical examinations many recent improvements developed outside of continental Europe are mentioned but briefly or not at all. For example, no reference is made to the use of flame photometry which has made practicable frequent determinations of sodium and potassium concentrations in blood and other body fluids. The bibliography consists almost exclusively of continental European articles of the decade beginning with 1930. There are an excellent glossary and tables of normal values.

In general this book is of greatest value to the advanced technician and fulfills the author's intention to serve as a laboratory manual and to acquaint the physician with some of the basic principles of many laboratory methods. The vast amount of information presented makes this book worthy of perusal and occasional use as a reference by English-speaking physicians.

R. C. H.

Gastroscopy: The Endoscopic Study of Gastric Pathology, 2nd edition. By RUDOLPH SCHINDLER. 443 pp. $20.00. *University of Chicago Press, Chicago, Illinois.*

The completely revised and second edition of this authoritative work is welcome indeed. It is befitting that it should emanate from the author who is the founder and the primary developer of modern gastroscopy.

Through its pages one will find traced the background and development of gastroscopy; the anatomy of the pharynx, esophagus and stomach with particular reference to gastros-

copy; the indications, contraindications, technique, limitations and dangers of the procedure presented in detail.

The relationship between gastroscopy and the x-rays are approached from the proper premise; if only one of these two methods is to prevail it is to be the latter; since both are available, they are complementary and not competitive. Thus, in carcinoma both methods are important; where one fails the other may be useful. Gastritis is best diagnosed by gastroscopy. Differential diagnosis between malignant and benign ulcers, is often correctly made by this procedure. Gastroscopic failures—readily acknowledged—will be further reduced with mechanical and optical improvements in instruments, techniques and more skillful examiners thereby reducing so-called "blind" areas and errors.

The minutiae and elaborateness in delineating gastroscopic technique and orientation appear to defeat the author's purpose for by this presentation these aspects are rendered more difficult for the beginner. Simpler descriptions will suffice. The gastroscopic differentiation between benign and malignant lesions needs further study in larger numbers of cases and by other methods before the extent of accuracy can be fully determined. There is a considerable body of opinion not wholly in agreement with Schindler as to the extent of dyspepsia due solely to gastritis. However, in relation to the excellence and comprehensiveness of the whole work, these examples of differences are of no striking moment.

Disturbances in gastric secretion, motility and circulation, the various gastric diseases including anomalies, foreign bodies and the derangement of the post-operative stomach are presented with clarity and detail.

There are more than a thousand references and an index of names of contributors, more than a hundred colored illustrations depicting the disorders confronting the gastroscopist in addition to scores of black and white drawings and photomicrographs.

Suffice it to say, the profession is indebted to Rudolph Schindler for the development of modern gastroscopy as well as for this remarkable treatise. It is essential to all working in this field.

The University of Chicago Press has presented this work in an excellent format worthy of its importance.

MOSES PAULSON

BOOKS RECEIVED FOR REVIEW

The Collected Papers of Adolf Meyer. Four Volume Set $30.00. Edited by EUNICE WINTERS. Vol. II, Psychiatry. With an Introduction by Sir DAVID K. HENDERSON, M.D. 676 pp. The Johns Hopkins Press, Baltimore, Md.

Diabetes Insipidus. By HARRY BLOTNER. Edited by HENRY A. CHRISTIAN. (Reprinted from Oxford Loose-Leaf Medicine). 200 pp. Oxford University Press, New York.

Diabetes Mellitus. Principles and Treatment. By GARFIELD G. DUNCAN. 289 pp. $5.75. W. B. Saunders Co., Philadelphia, Pa.

Diseases of the Heart and Circulation. By PAUL WOOD. 589 pp. $12.50. J. B. Lippincott Co., Philadelphia, Pa.

Electroencephalography in Clinical Practice. By ROBERT S. SCHWAB. 195 pp. $6.50. W. B. Saunders Co., Philadelphia, Pa.

Handbook of Pediatric Medical Emergencies. By ADOLPH G. DESANCTIS and CHARLES VARGA. 284 pp. $5.00. The C. V. Mosby Co., St. Louis, Missouri.

Herzschalldiagnostik in Klinik und Praxis. Eine Einführung in den Praktischen Gebrauch der Herzschallschreibung. By J. SCHMIDT-VOIGT. 116 pp. Kartoniert DM 9.60. Georg Thieme Verlag, Stuttgart, Germany.

A History of Nursing, 2nd edition. By GLADYS SELLEW and C. J. NUESSE. 439 pp. $3.75. The C. V. Mosby Co., St. Louis, Missouri.

Immunology, 3rd edition. By NOBLE PIERCE SHERWOOD. 731 pp. $8.00. The C. V. Mosby Co., St. Louis, Missouri.

Leitfaden der Blutmorphologie. By LYDIA SCHUDEL. 50 pp. Kartoniert DM 14.80 ($3.50). Georg Thieme Verlag, Stuttgart, Germany.

Maternal Care and Mental Health. World Health Organization Monograph Series No. 2. 179 pp. $2.00. World Health Organization, Palais de Nations, Geneva.

Medicine of the Year 1951. Editorial Direction of JOHN B. YOUMANS. 298 pp. $5.00. J. B. Lippincott Co., Philadelphia, Pa.

The Neuroses. Diagnosis and Management of Functional Disorders and Minor Psychoses. By WALTER C. ALVAREZ. 667 pp. $10.00. W. B. Saunders Co., Philadelphia, Pa.

Patterns of Disease. On a Basis of Physiologic Pathology. By Frank L. Apperly. 456 pp. $8.00. J. B. Lippincott Co., Philadelphia, Pa.

Personnel Administration in Public Health Nursing. By WILLIAM BRODY. 209 pp. $3.25. The C. V. Mosby Co., St. Louis, Missouri.

Proceedings of the Forum Sessions; Thirty-Sixth Clinical Congress of the American College of Surgeons, Boston, Massachusetts, October, 1950. Surgical Forum Committee, Owen H. Wangensteen, Warren H. Cole, Robert E. Gross, Michael L. Mason, Carl A. Moyer, and I. S. Ravdin. 665 pp. $10.00. W. B. Saunders Co., Philadelphia, Pa.

A Text-Book of X-ray Diagnosis, 2nd edition, Vol. 2. By British authors in four volumes. Edited by S. Cochrane Shanks and Peter Kerley. 702 pp. $15.00. W. B. Saunders Co., Philadelphia, Pa.

Vitamin E: Annotated Bibliography 1940 to 1950. By PHILIP L. HARRIS AND WILMA KUJAWSKI. 184 pp. $3.00. The National Vitamin Foundation, New York.